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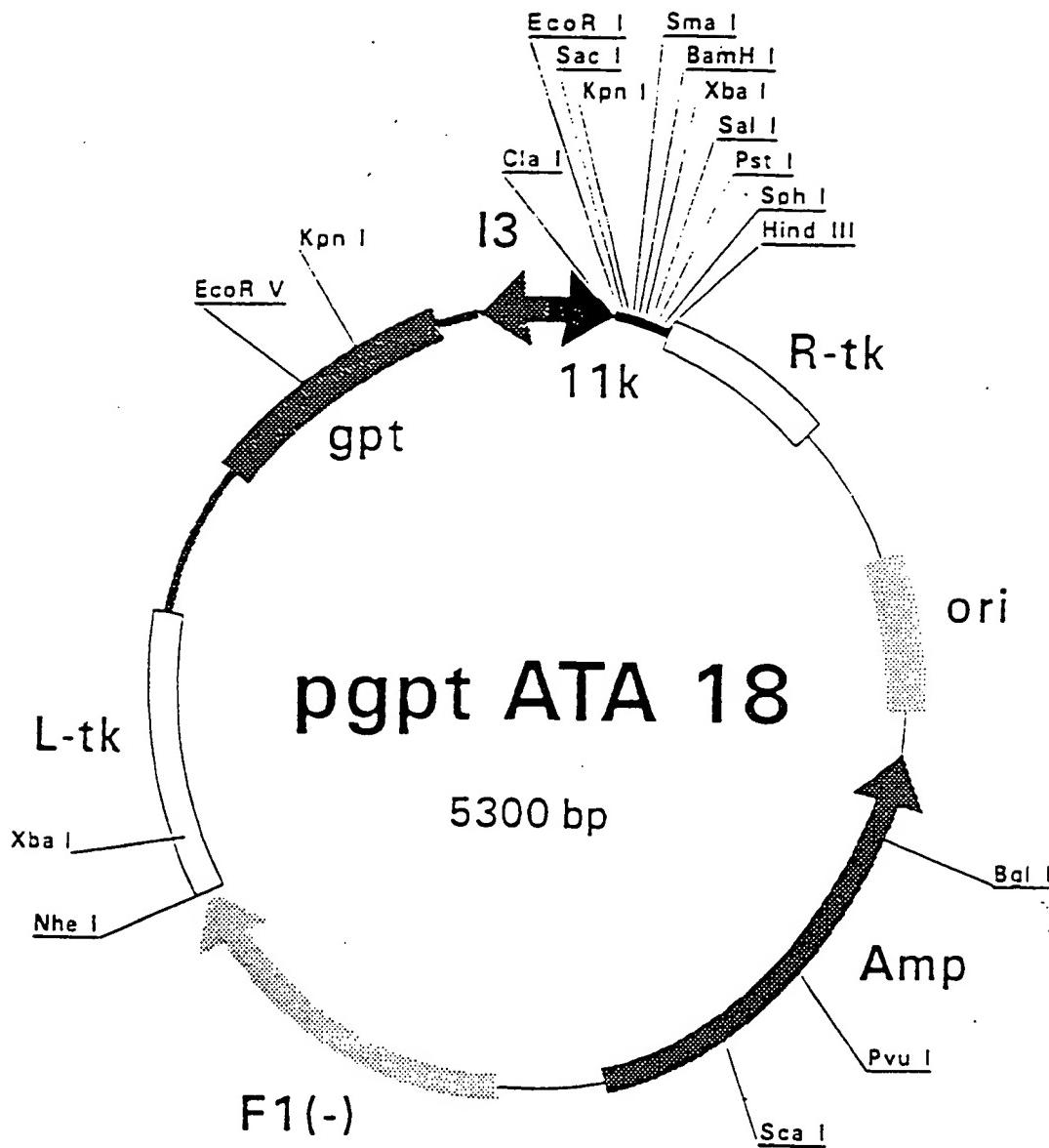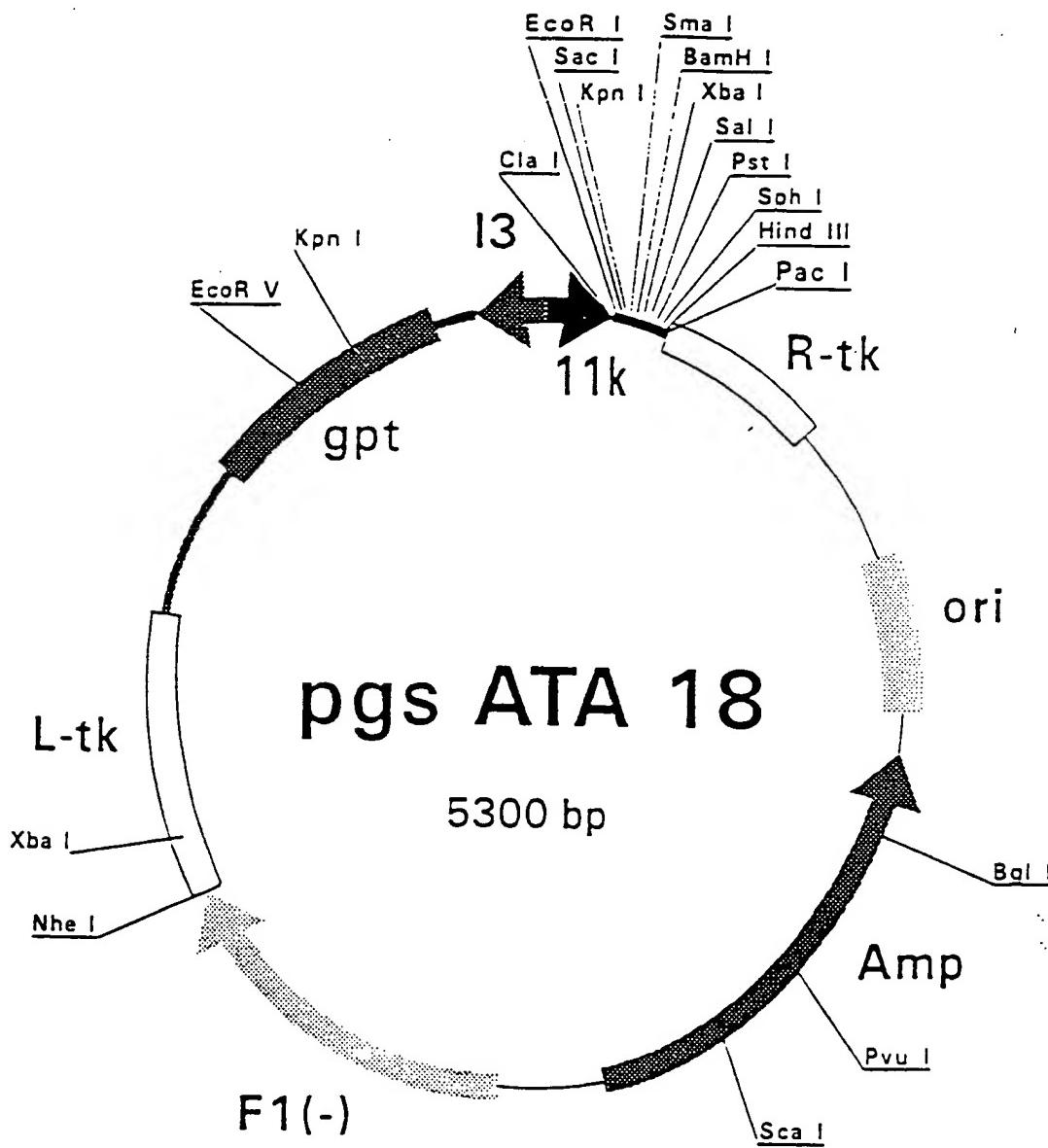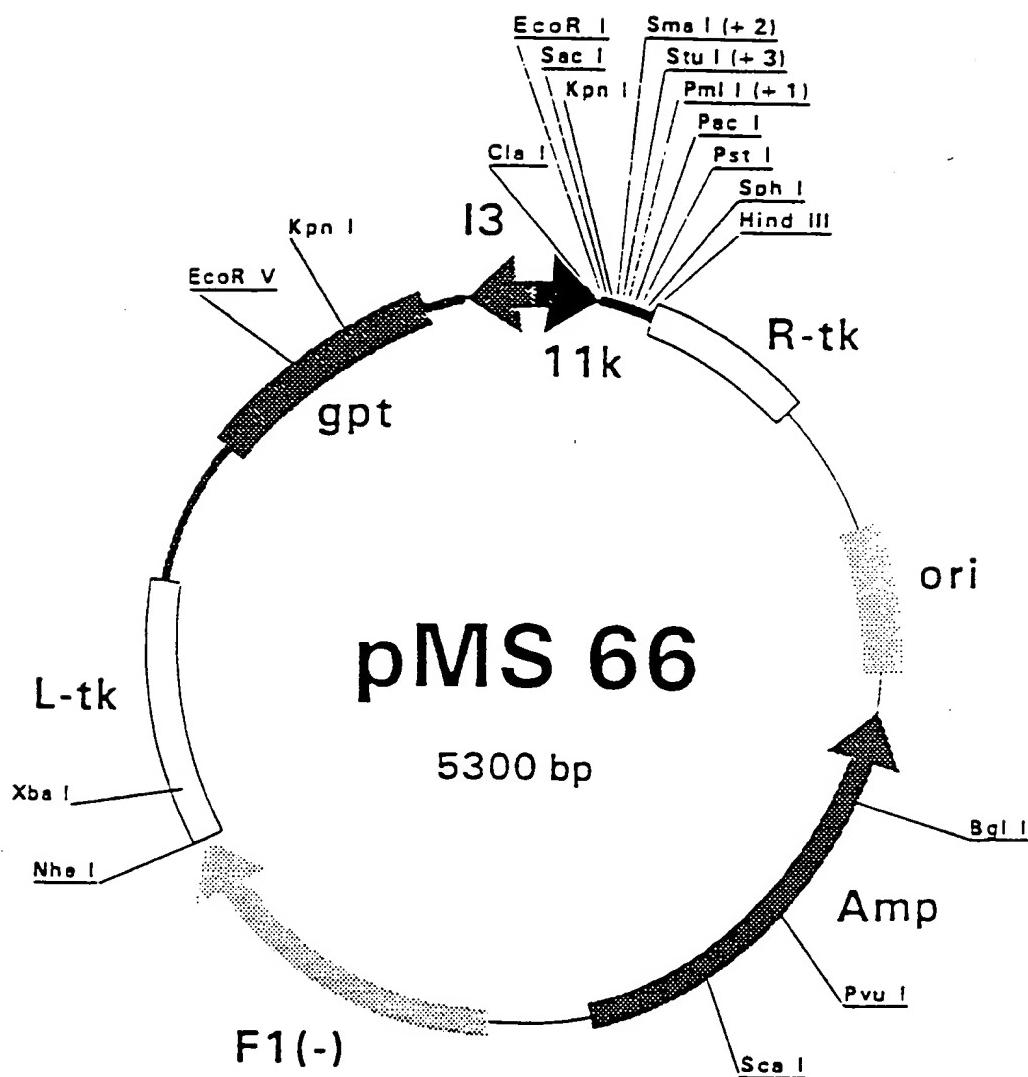
FIGURE 1
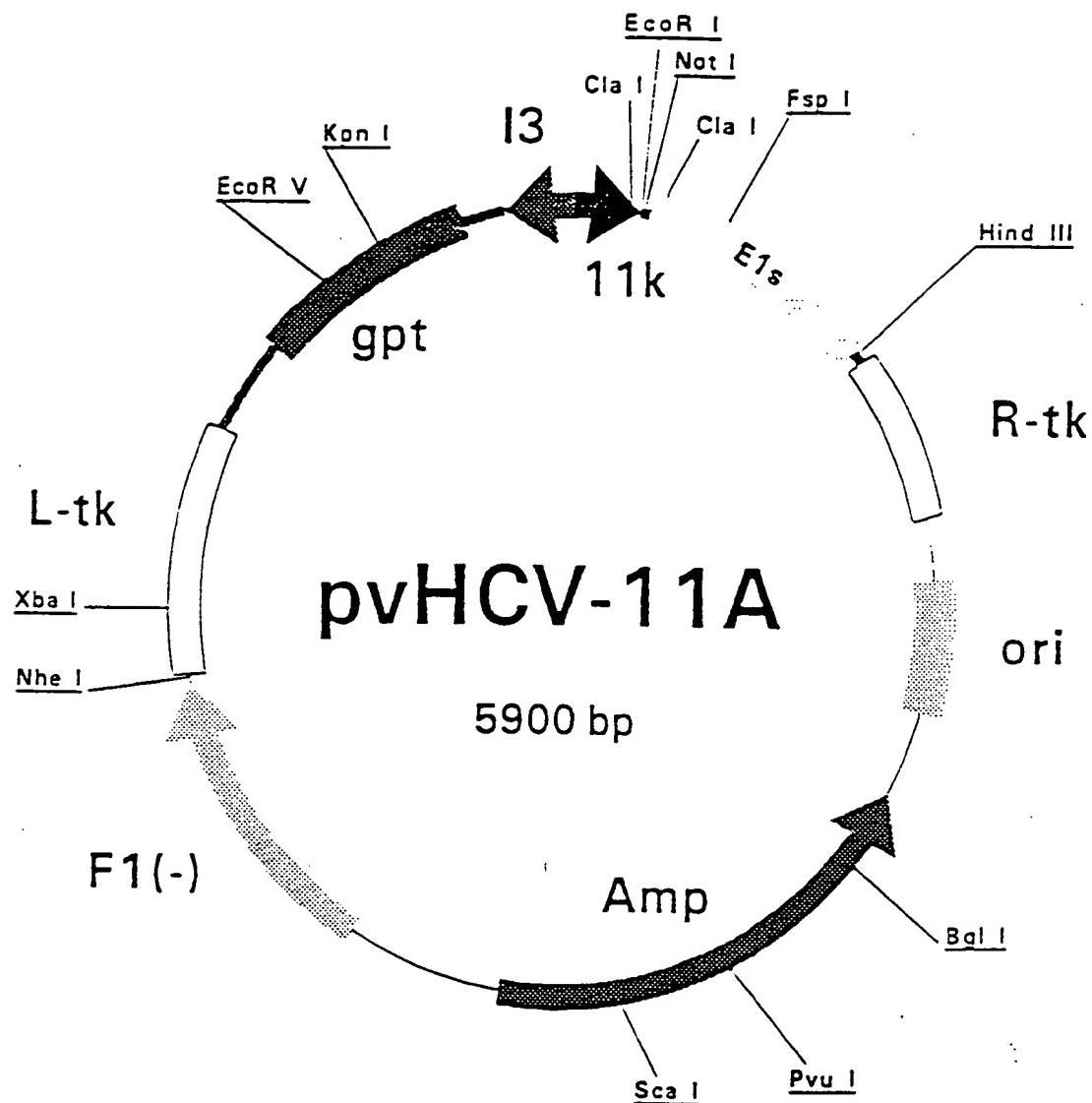
FIGURE 2

FIGURE 3

FIGURE 4

## Anti-E1 levels in NON-responders to IFN treatment
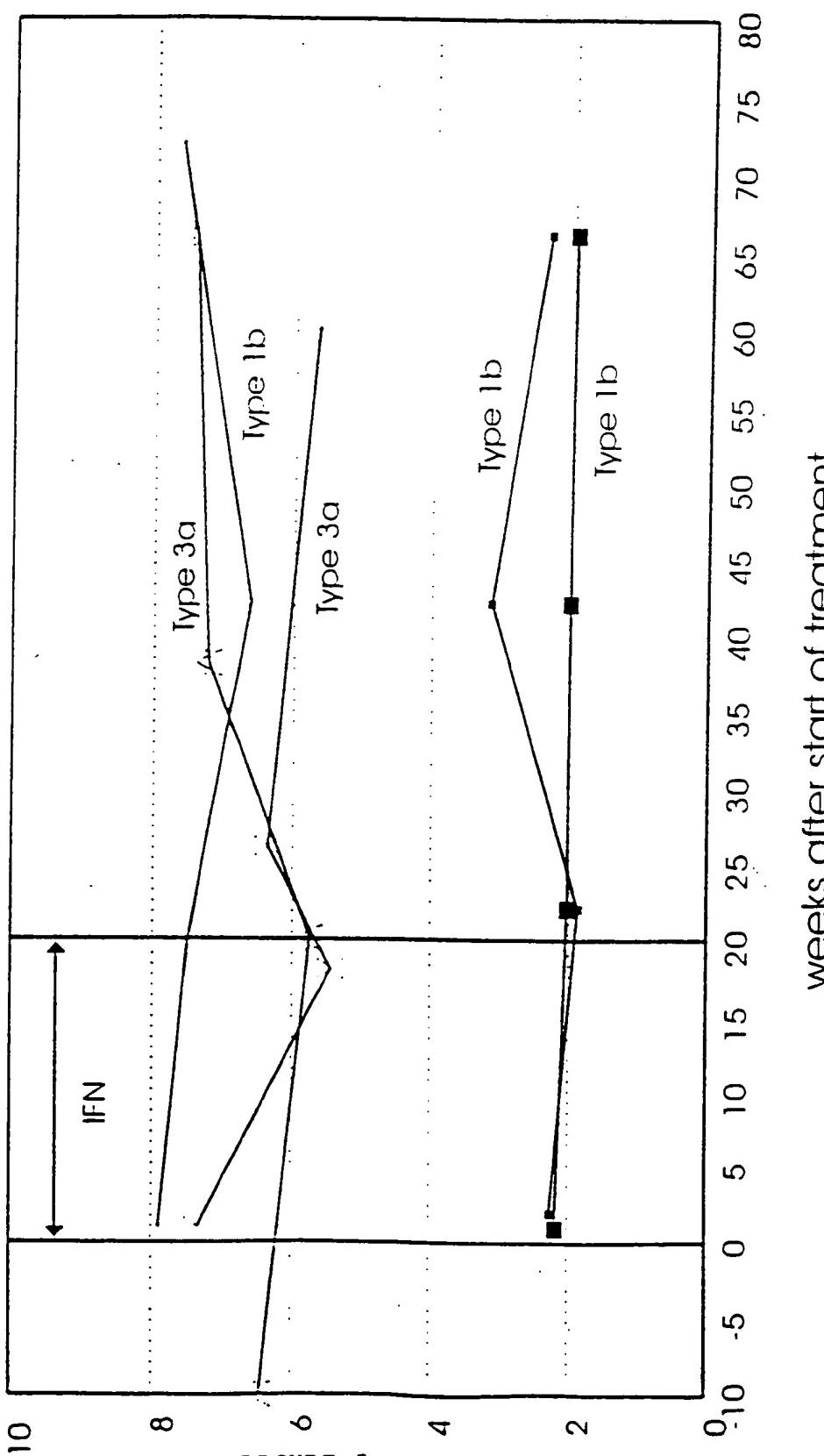
Series 1



FIGURE 5

Anti-E1 levels in RESPONDERS to IFN treatment
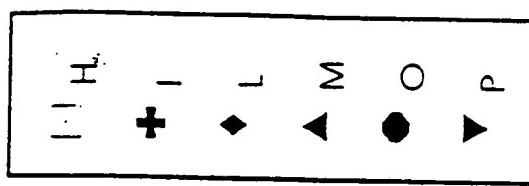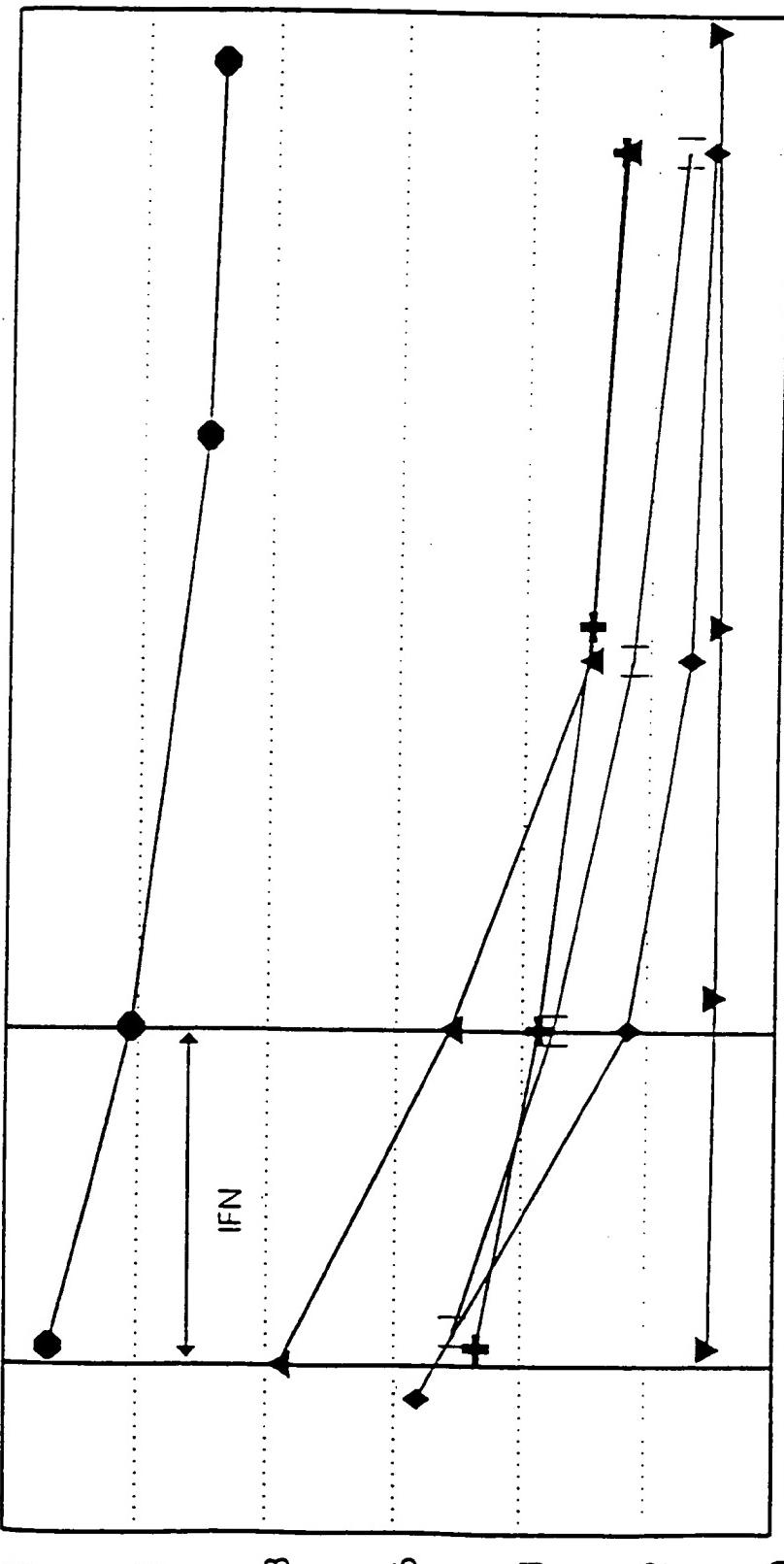
SERIES 1

Anti-E1 levels in patients with COMPLETE response to IFN

SERIES 2
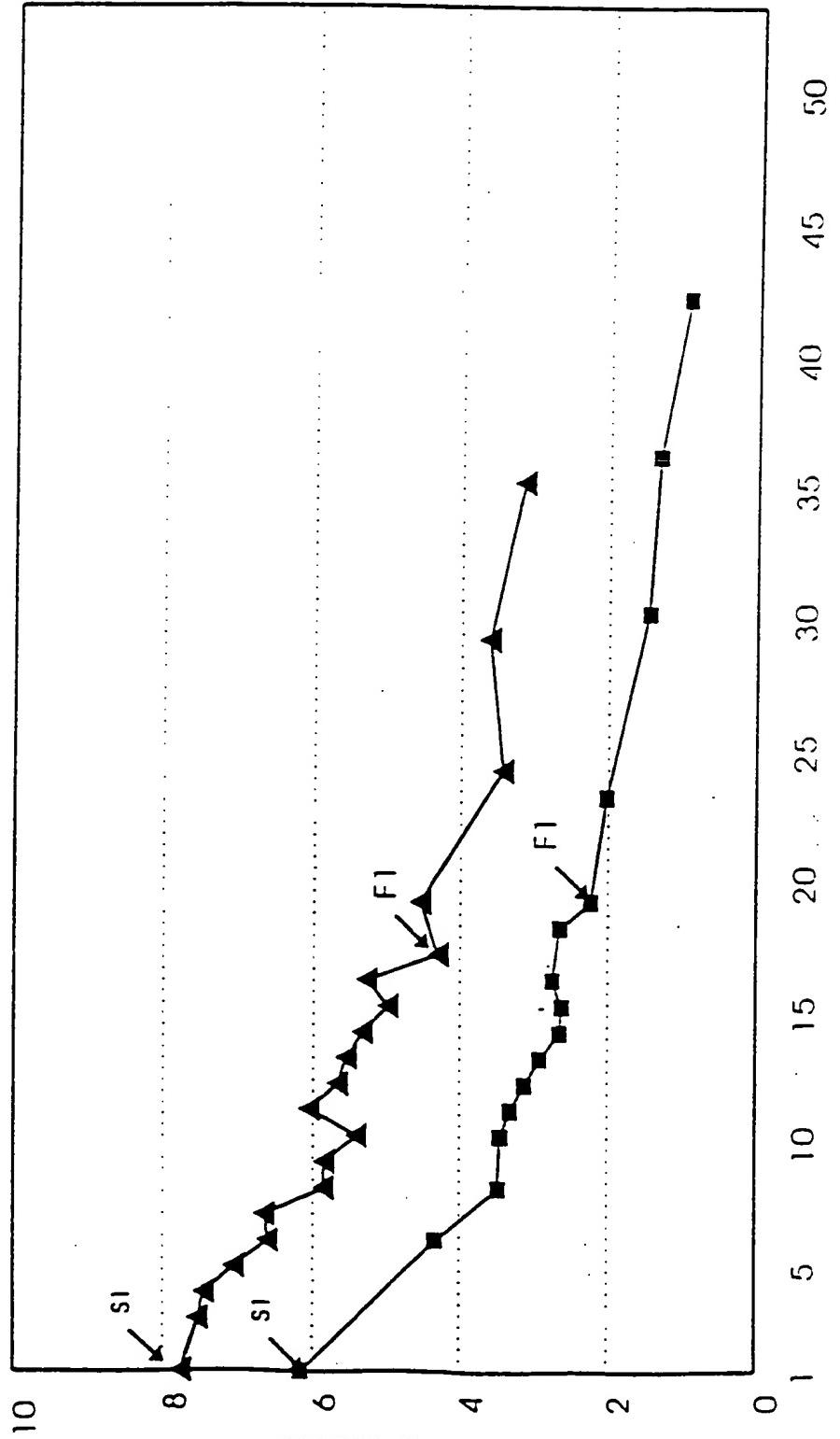
FIGURE 7

Anti-E1 levels in INCOMPLETE responders to IFN treatment

SERIES 2

Legend:
- A 1b NR
- B 1b NR
- C 3a RR
- F 1b PR

S: start of treatment
F: finish of treatment

months after start of treatment

FIGURE 8

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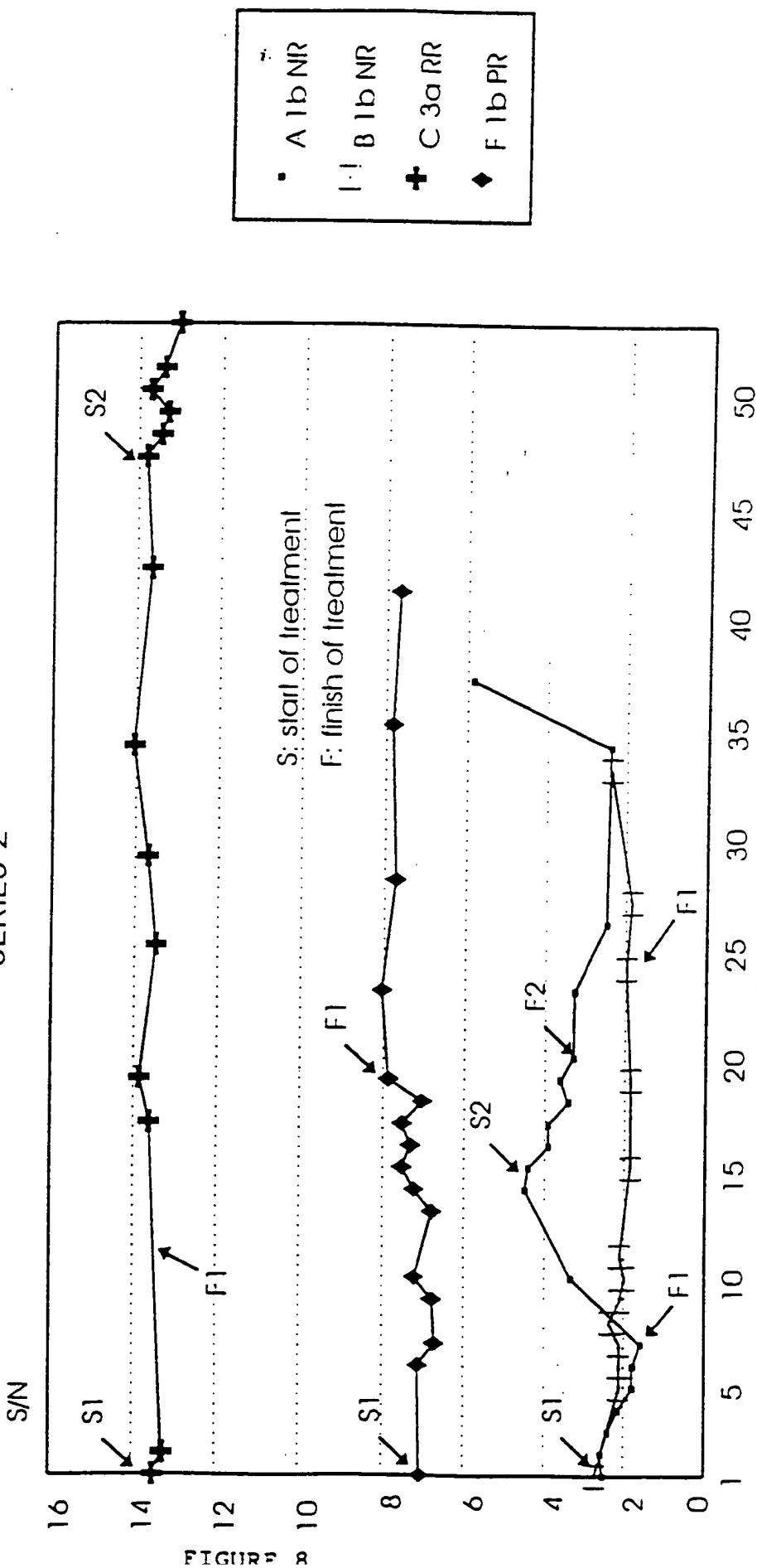
Anti-E2 levels in NON-RESPONDERS to IFN treatment

SERIES 1

weeks after start of treatment

FIGURE 9
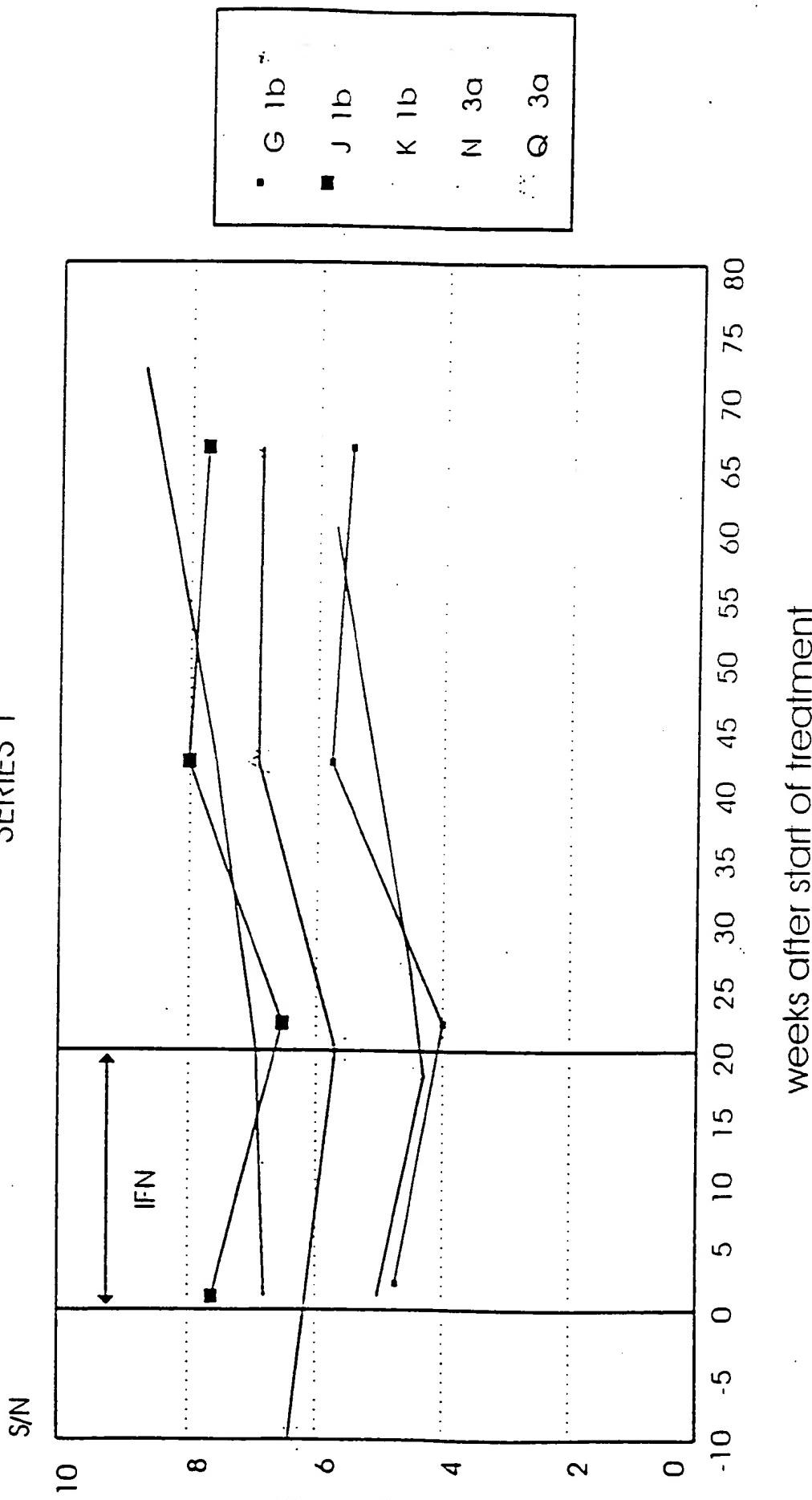
Anti-E2 levels in RESPONDERS to IFN treatment
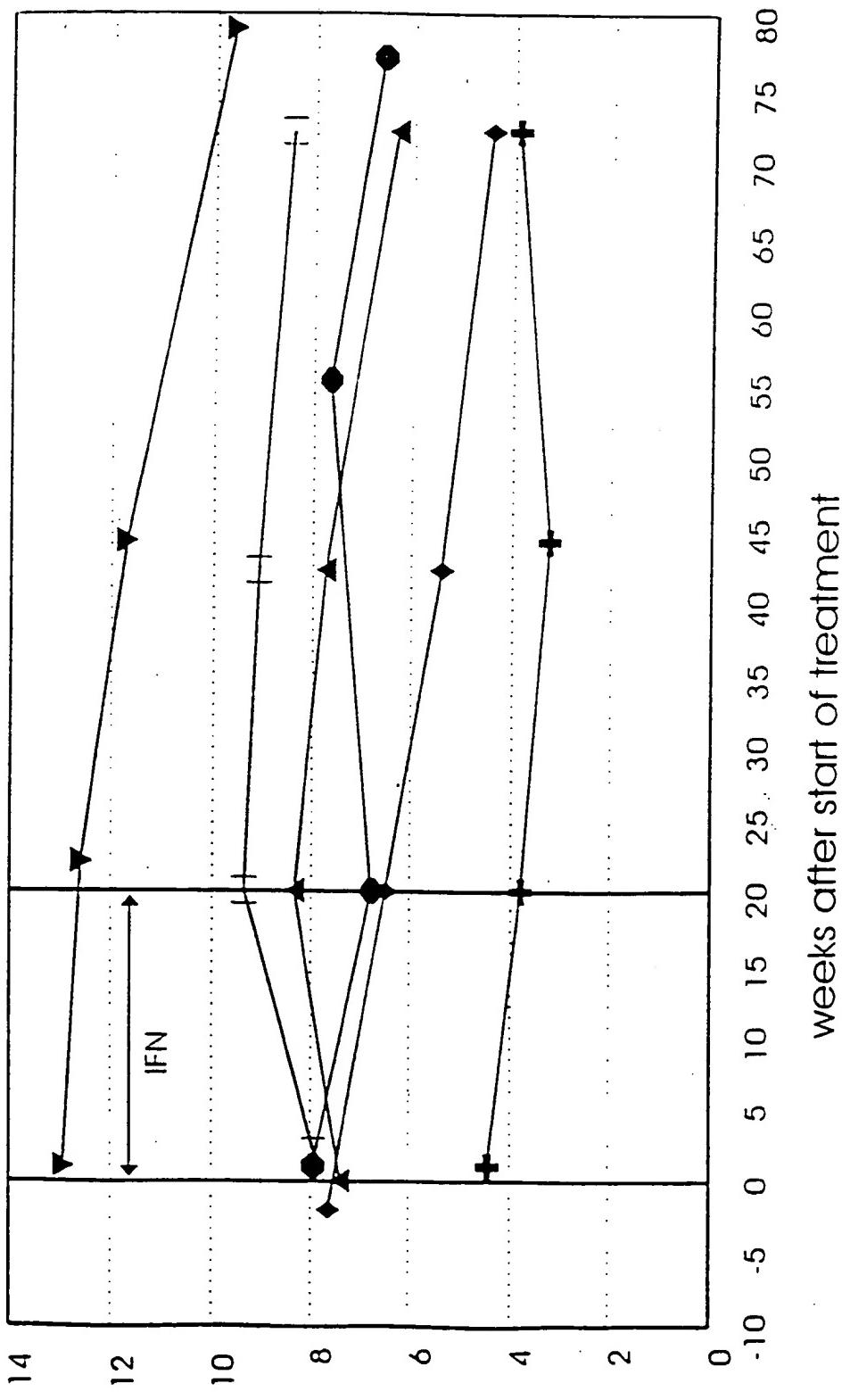
SERIES 1


FIGURE 13

Anti-E2 levels in INCOMPLETE responders to IFN treatment
SERIES 2

FIGURE 11

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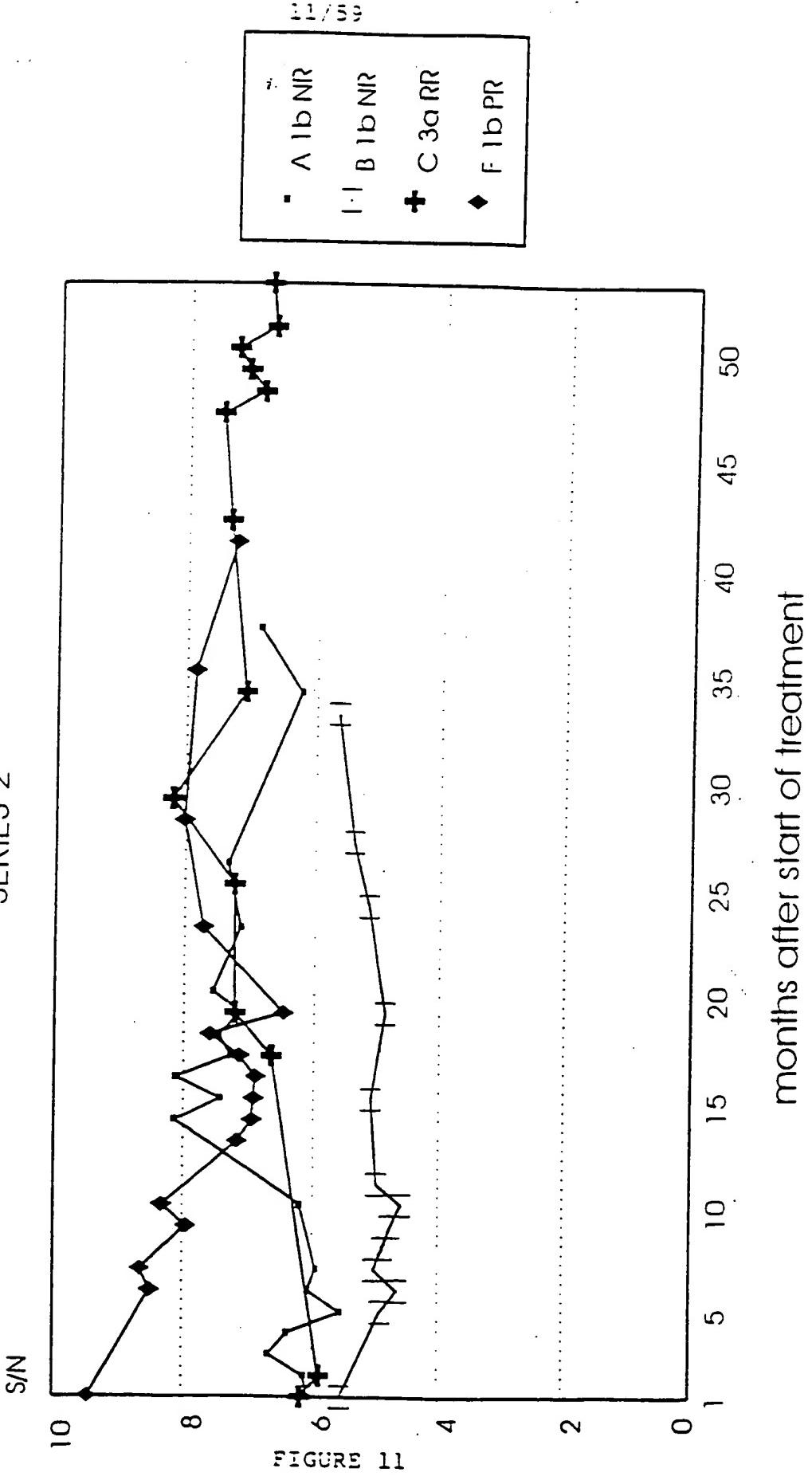
Anti-E2 levels in COMPLETE responders to IFN treatment

SERIES 2

■ D 3a CR
▲ E 3a CR

F : finish of treatment

months after start of treatment
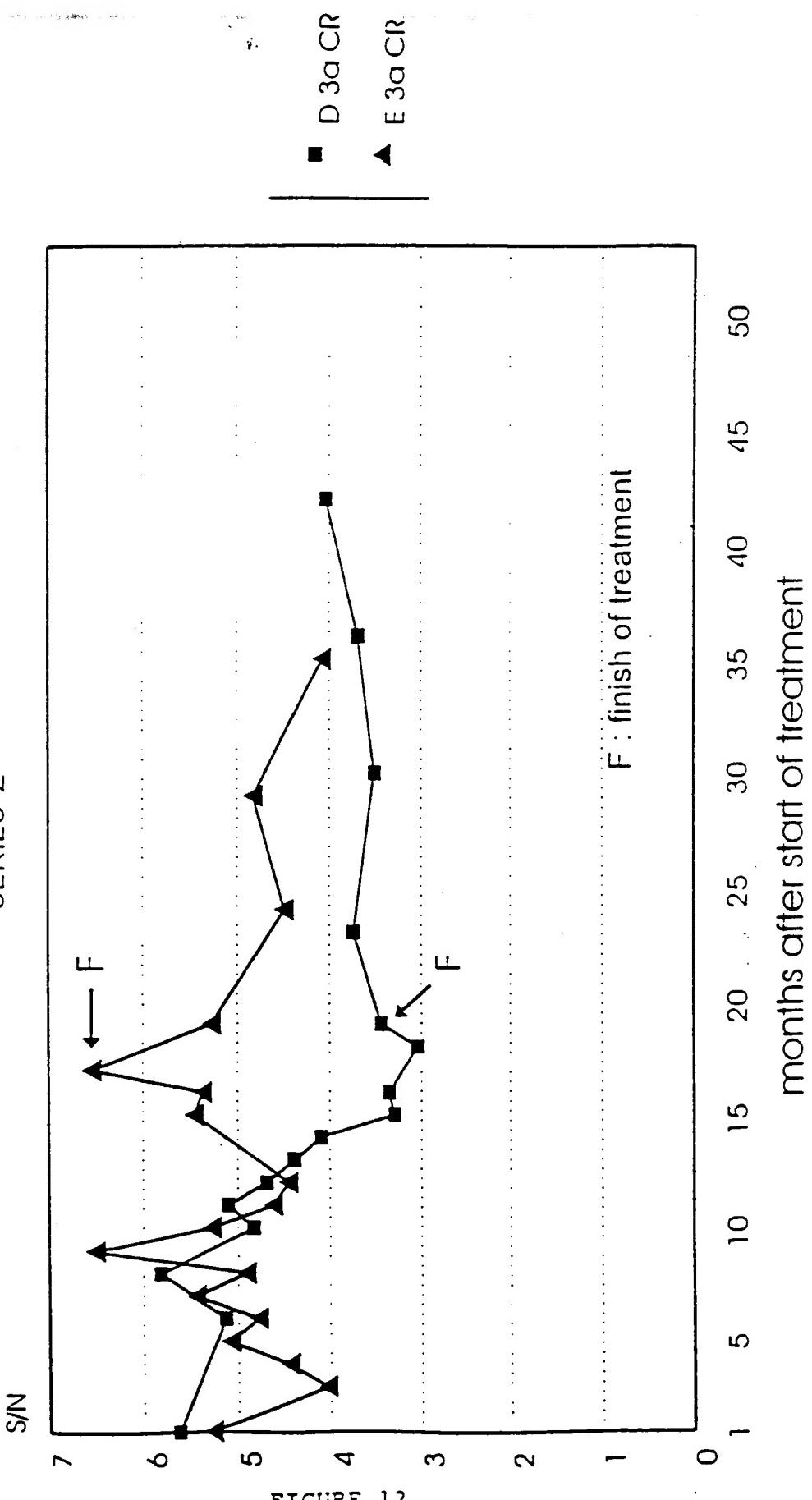
FIGURE 12

FIGURE 13

Human anti-E1 reactivity competed with peptides
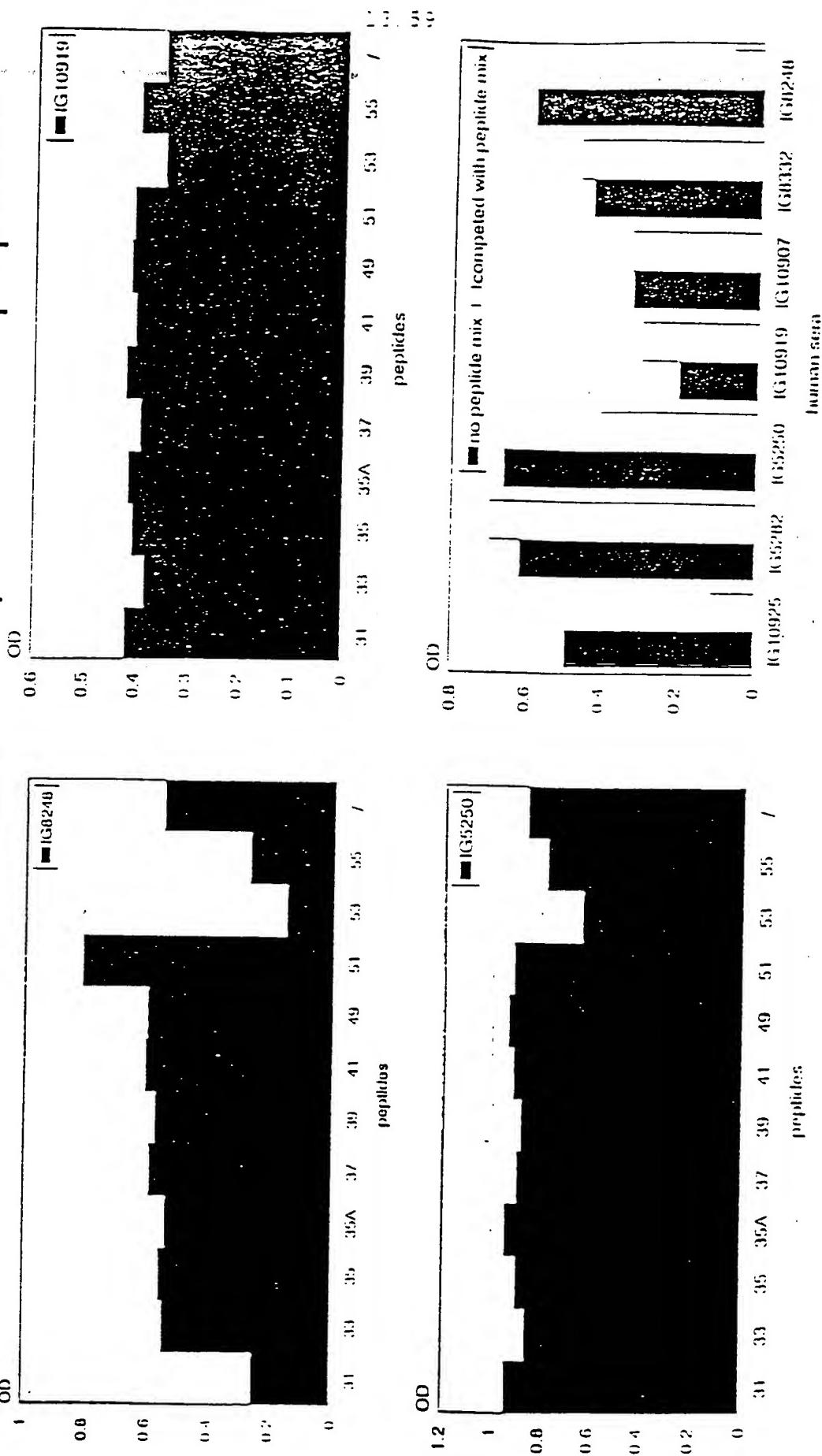
FIGURE 14

Competition of reactivity of anti-E1 Mabs with peptides

Anti-E1 (epitope 1) levels in NON-RESPONDERS to IFN treatment
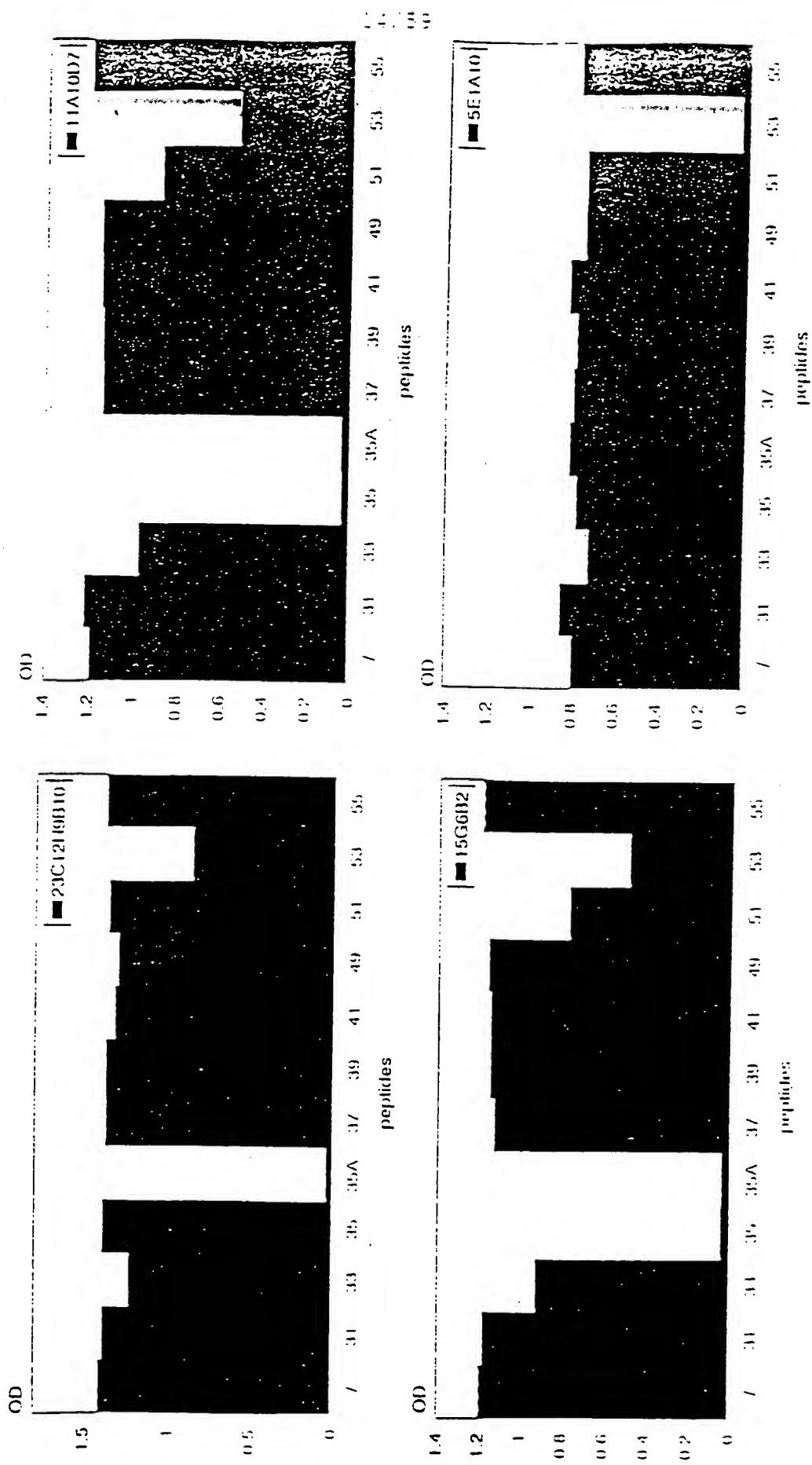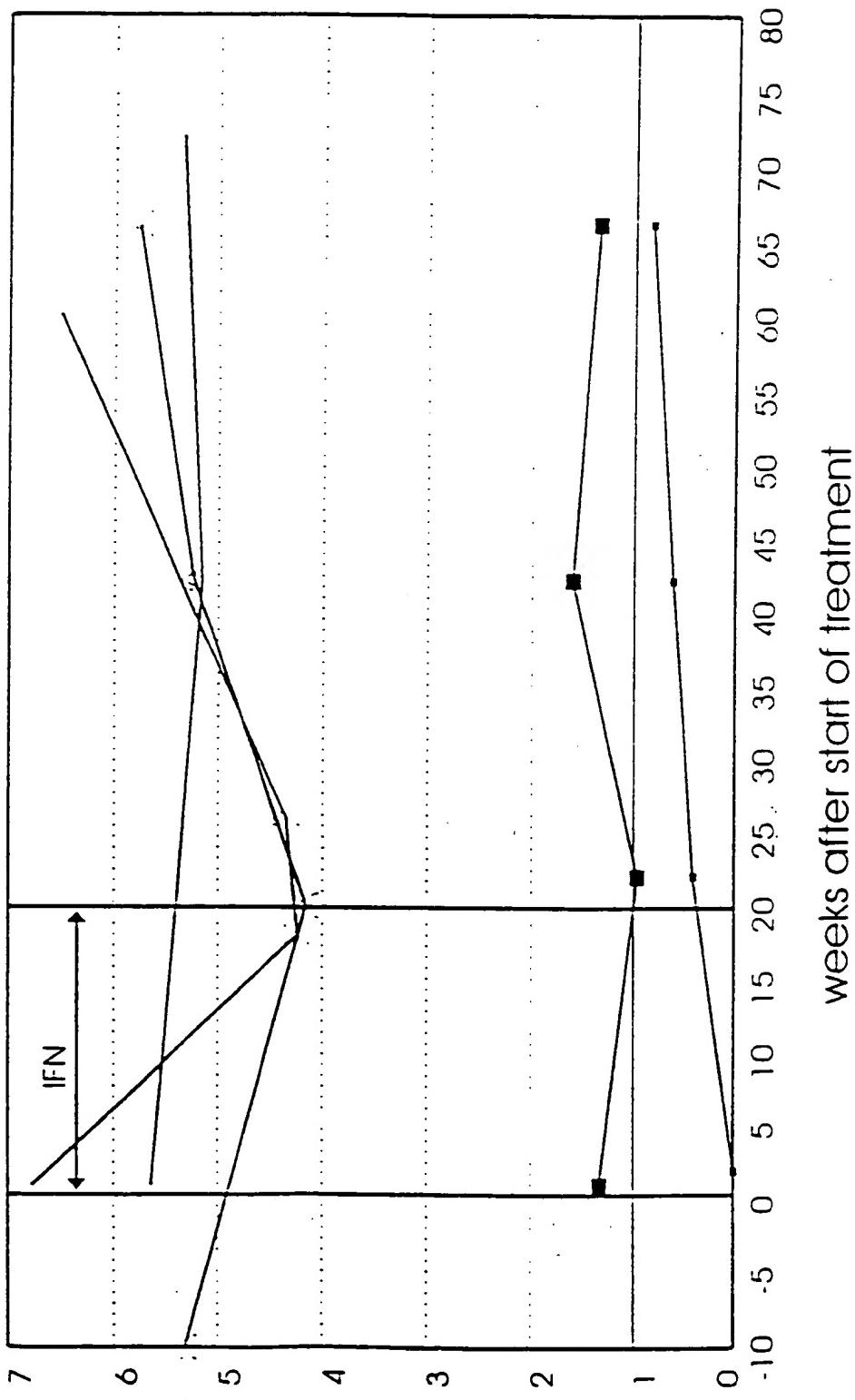
SERIES 1

FIGURE 15

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Anti-E1 (epitope 1) levels in RESPONDERS to IFN treatment
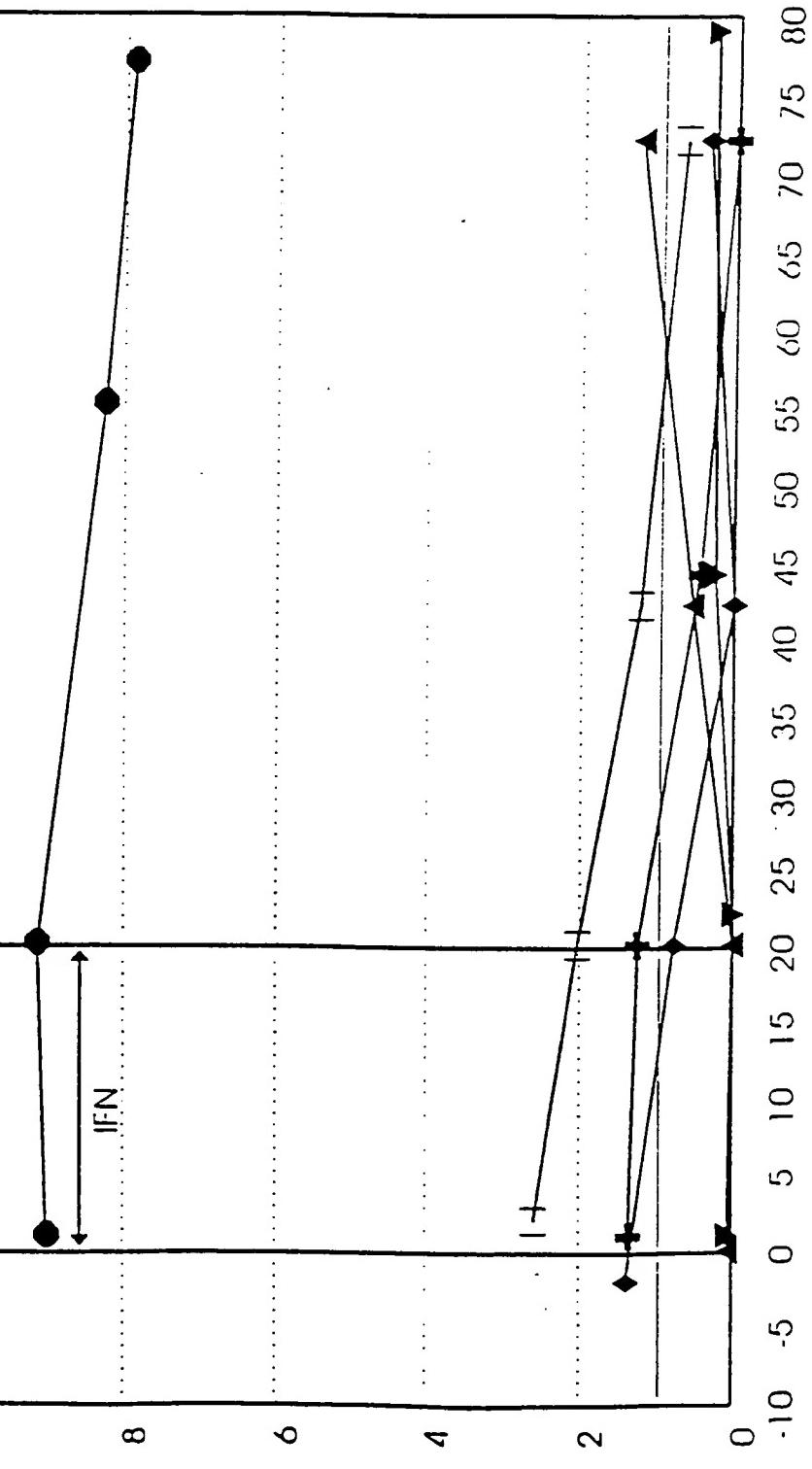
SERIES 1

weeks after start of treatment

Legend:
- □ H 1b
- + I 1b
- ◆ L 1b
- ◀ M 1b
- ● O 3a
- ▶ P 1b

anti-E1 (epitope 2) levels in NON-RESPONDERS to IFN treatment

SERIES 1

S/N

weeks after start of treatment

IFN

G 1b

J 1b

K 1b

N 3a

Q 3a

Anti-E1 (epitope 2) levels in RESPONDERS to IFN treatment

SERIES 1

Legend	
□	H 1b
+	I 1b
◆	L 1b
◄	M 1b
●	O 3a
►	P 1b

weeks after start of treatment

S/N
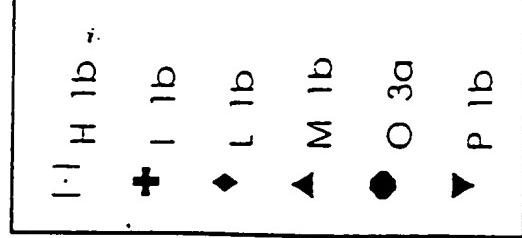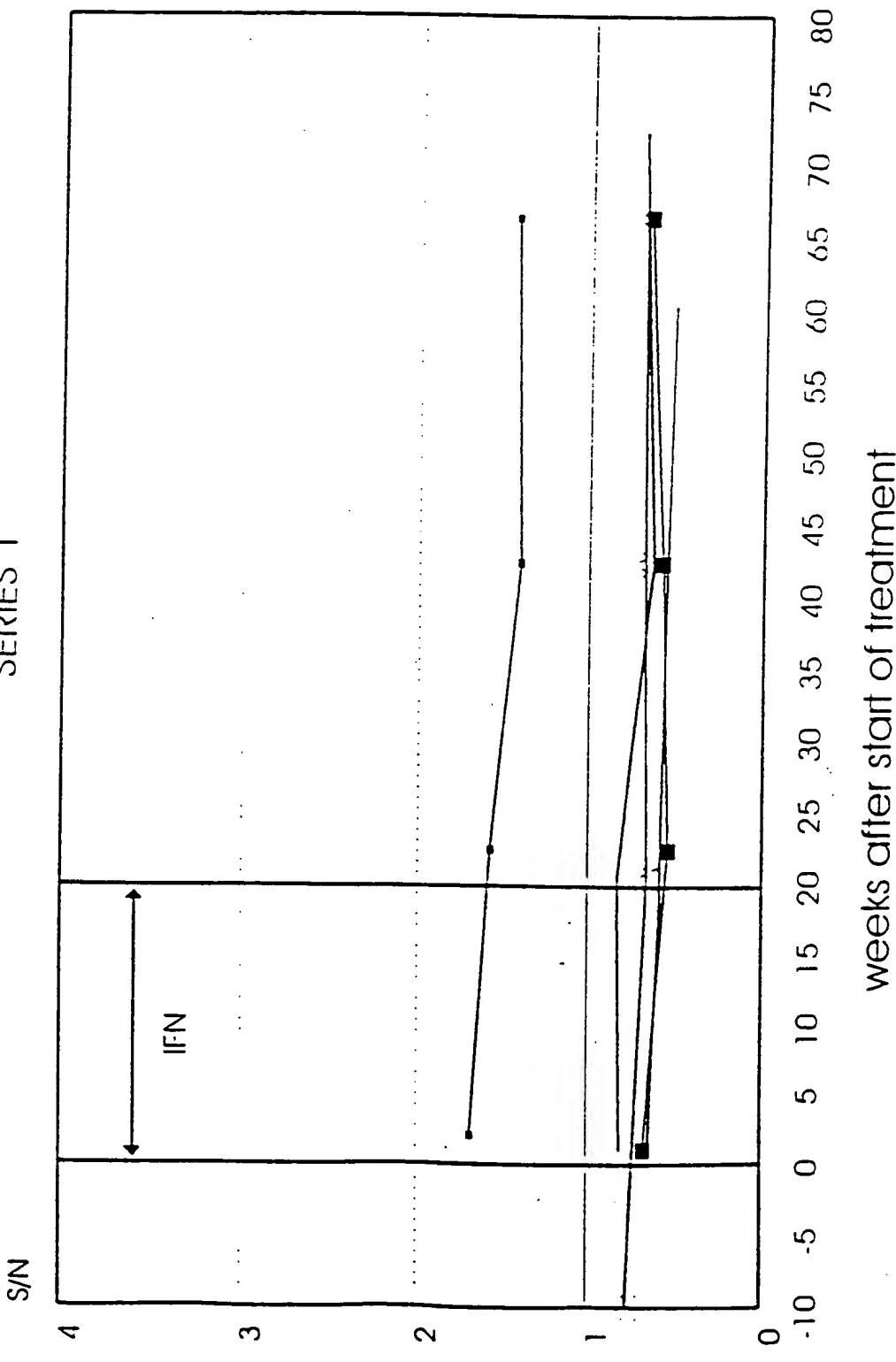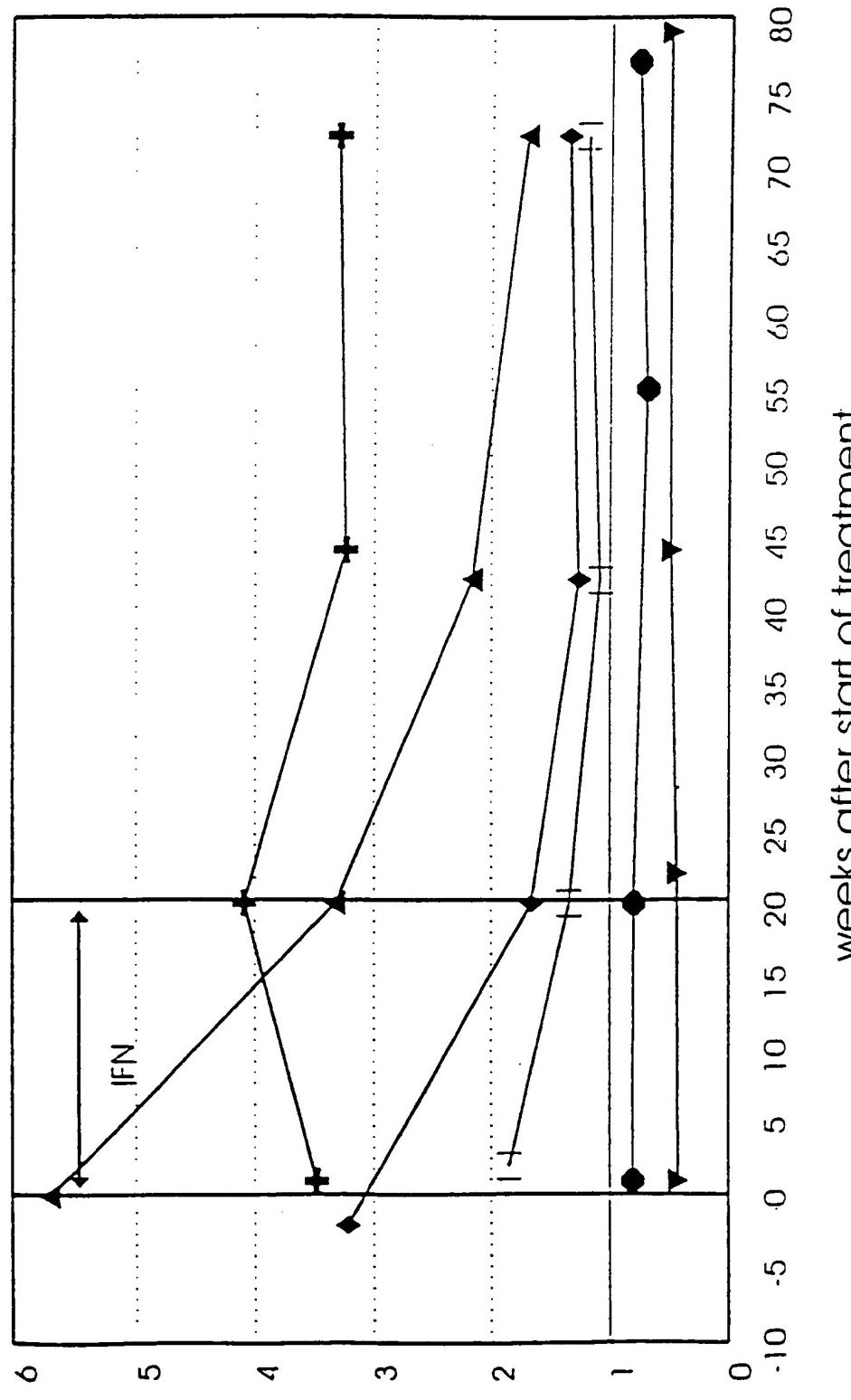
FIGURE 19

Competition of reactivity of anti-E2 Mabs with peptides
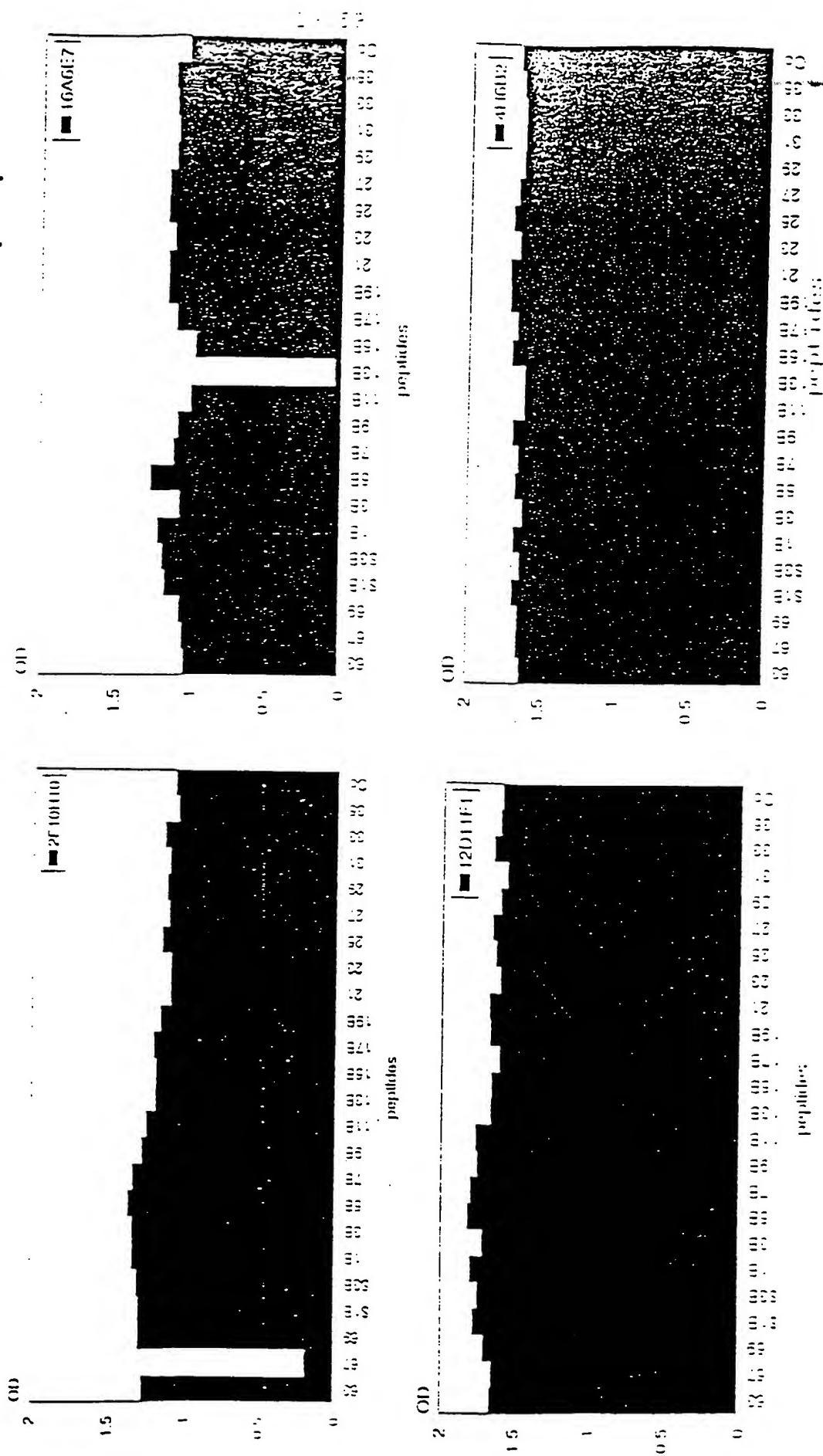
FIGURE 20

Human anti-E2 reactivity competed with peptides
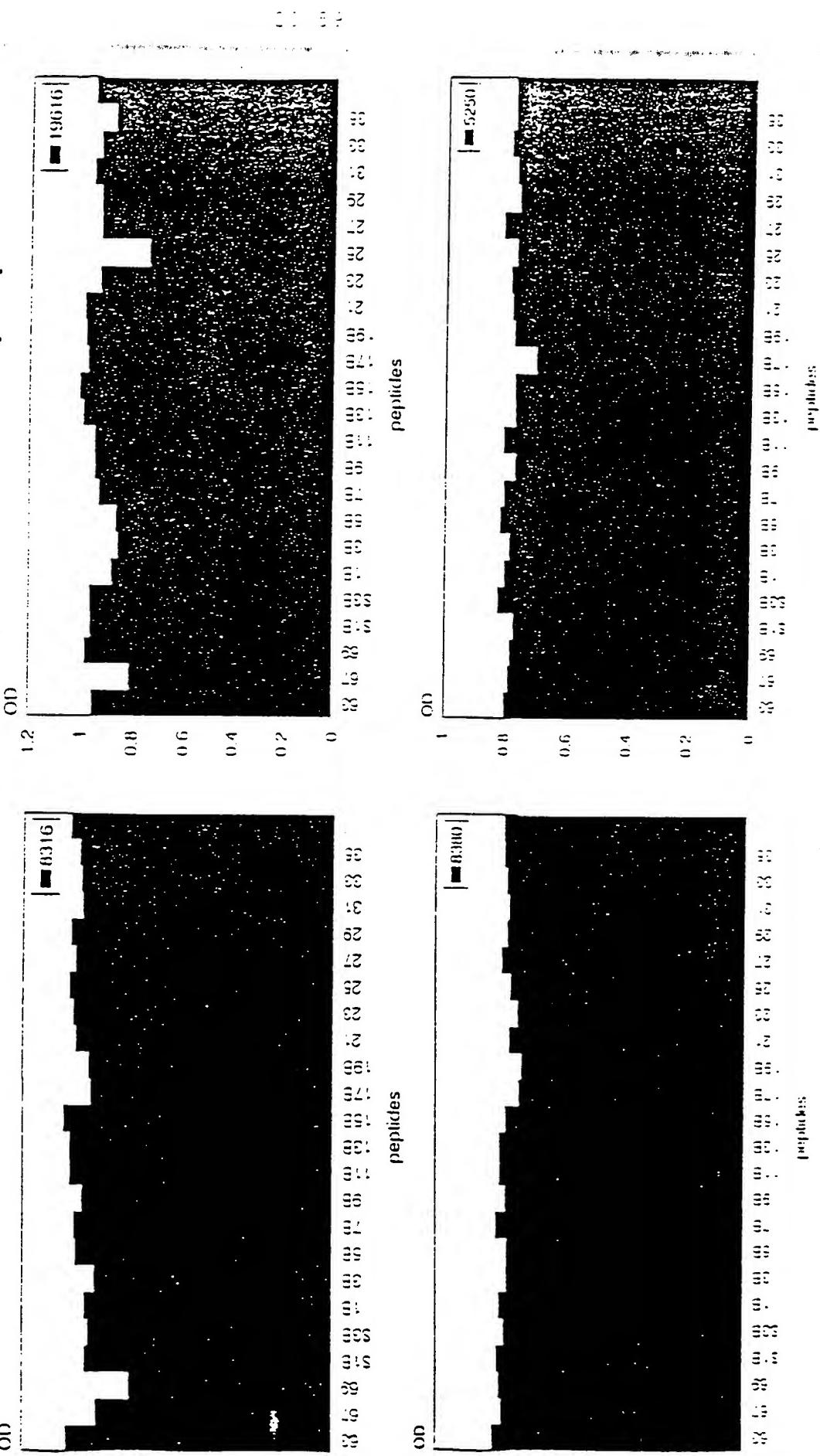
Figure 21

5' GGCATGCAAGCTTAATTAATT3' (SEQ ID NO 1)

3'ACGTCCGTACGTTCGAATTAATTAATCGA5' (SEQ ID NO 94)

5'CCGGGGAGGCCTGCACGTGATCGAGGGCAGACACCATCACCACCATCACTAATAGT
TAATTAACTGCA 3' (SEQ ID NO 2)

3'CCTCCGGACGTGCACTAGCTCCCGTCTGTGGTAGTGGTGGTAGTGATTATCAATTAATTG

5' (SEQ ID NO 95)

SEQ ID NO 3 (HCCI9A)

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CCAACTCAAGCATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGT
GCCCTGCGTTCGGGAGAACAACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTC
GCAGCTAGGAACGCCAGCGTCCCCACCACGACAATACGACGCCACGTCGATTTGCTCG
TTGGGGCGGCTGCTCTCTGTTCCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTC
CTCGTCTCCCAGCTGTTCACCATCTCGCCTCGCCGGCATGAGACGGTGCAGGACTGCA
ATTGCTCAATCTATCCCGGCCACATAACAGGTCACCGTATGGCTTGGGATATGATGAT
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SEQ ID NO 5 (HCCI10A)

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SEQ ID NO 7 (HCCI11A)

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GCGGCCAGGAACGCCAGCGTCCCCACAACGACAATACGACGCCACGTCGATTTGCTC
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SEQ ID NO 11 (HCCI13A)

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GAACTGGTAATAG

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SEQ ID NO 15 (HCPr51)
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SEQ ID NO 16 (HCPr52)
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SEQ ID NO 17 (HCPr53)
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SEQ ID NO 18 (HCPr54)
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SEQ ID NO 19 (HCPr107)
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SEQ ID NO 20 (HCPr108)

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SEQ ID NO 21 (HCCl37)

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SEQ ID NO 23 (HCCl38)

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SEQ ID NO 25 (HCCl39)

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SEQ ID NO 31 (HCCI63)

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SEQ ID NO 34 (HCPr72)

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SEQ ID NO 35 (HCCL22A)

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SEQ ID NO 37 (HCCI41)

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SEQ ID NO 39 (HCCI42)

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SEQ ID NO 41 (HCCI43)

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CGTCTCAGGTGTGCGGTCCAGTGTATTGCTTCACCCCGAGCCCTGTTGTGGTGGGGAC
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SEQ ID NO 43 (HCCI44)

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SEQ ID NO 45 (HCCL64)

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SEQ ID NO 47 (HCCI65)

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SEQ ID NO 49 (HCCI66)

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GCTGCCTGGTACATCAAGGGCAGGCTGGTCCCTGGTGCGGCATACGCCTTCTATGGCG
TGTGGCCGCTGCTCCTGCTTCTGCTGGCCTTACCACCACGAGCTTATGCCTAGTAA
```

Figure 22

OD measured at 450 nm
construct

Fraction	volume	dilution	39 Type Ib	40 Type Ib	62 Type 3a	63 Type 3a
START	23 ml	1/20	2.517	1.954	1.426	1.142
FLOW THROUGH	23 ml	1/20	0.057	0.085	0.176	0.120
1	0.4 ml	1/200	0.102	0.051	0.048	0.050
2			0.396	0.550	0.090	0.067
3			2.627	2.603	2.481	2.372
4			3	2.967	3	2.694
5			3	2.810	2.640	2.154
6			2.694	2.499	1.359	1.561
7			2.405	2.481	0.347	1.390
8			2.176	1.970	1.624	0.865
9			1.461	1.422	0.887	0.604
10			1.256	0.926	0.543	0.519
11			0.981	0.781	0.294	0.294
12			0.812	0.650	0.249	0.199
13			0.373	0.432	0.239	0.209
14			0.653	0.371	0.145	0.184
15			0.441	0.348	0.151	0.151
16			0.321	0.374	0.098	0.106
17			0.525	0.186	0.099	0.108
18			0.351	0.171	0.083	0.090
19			0.192	0.164	0.084	0.087

34/59

OD 450 nm

FRACTIES

POOL

Type 1b (construct 39)
Type 1b (construct 40)
Type 3a (construct 62)
Type 5a (construct 63)
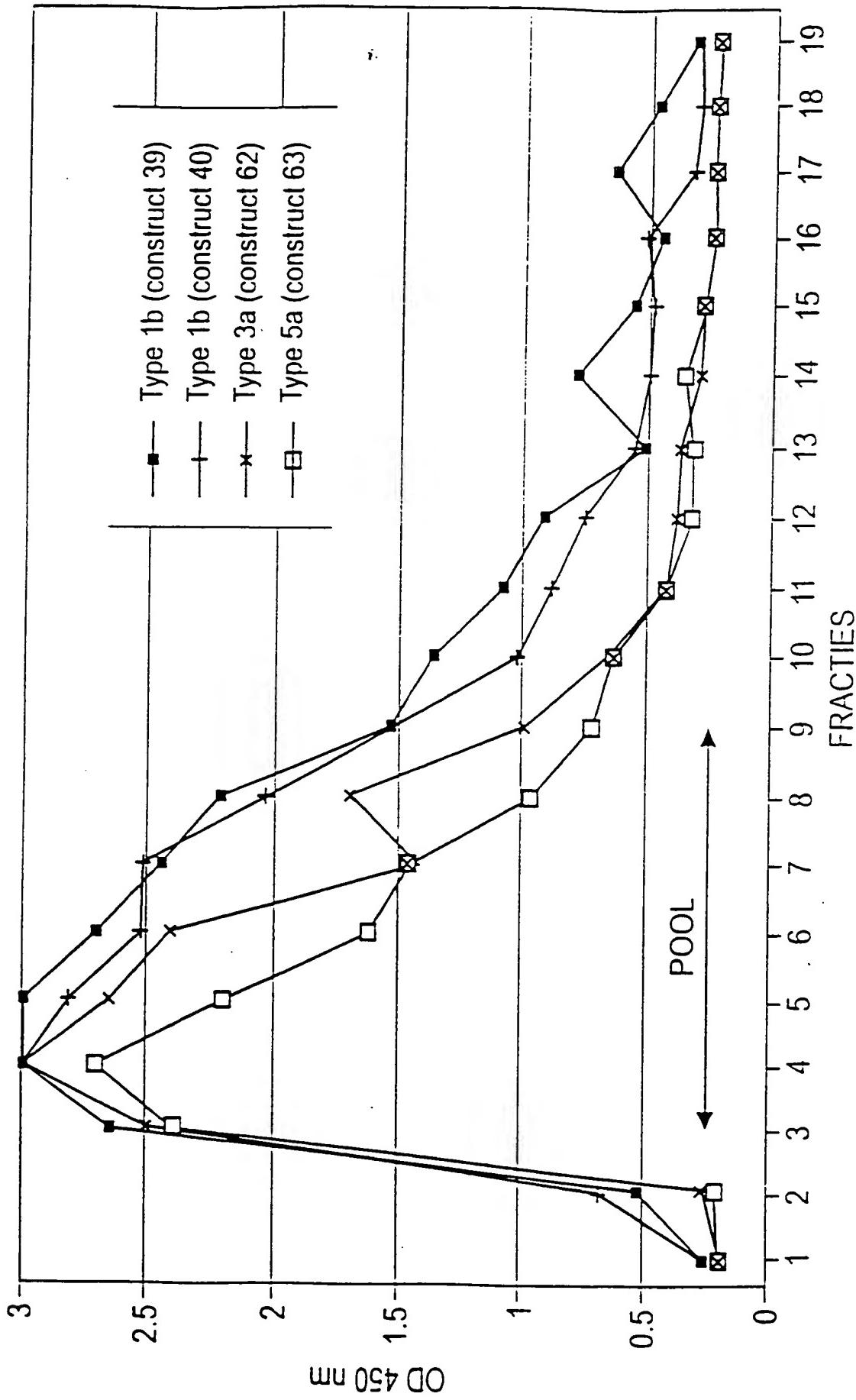
35/59 Figure 24

			OD measured at 450 nm construct			
Fraction	volume	dilution	39 Type 1b	40 Type 1b	62 Type 3a	63 Type 5a
20	250 µl	1/200	0.072	0.130	0.096	0.051
21			0.109	0.293	0.084	0.052
22			0.279	0.249	0.172	0.052
23			0.093	0.151	0.297	0.054
24			0.080	0.266	0.438	0.056
25			0.251	0.100	0.457	0.048
26			3	1.649	0.722	0.066
27			3	3	2.526	0.689
28			3	3	3	2.345
29			3	3	2.849	2.580
30			2.227	1.921	1.424	1.333
31			0.263	0.415	0.356	0.162
32			0.071	0.172	0.154	0.064
33			0.103	0.054	0.096	0.057
34			0.045	0.045	0.044	0.051
35			0.043	0.047	0.045	0.046
36			0.045	0.045	0.049	0.040
37			0.045	0.047	0.046	0.048
38			0.046	0.048	0.047	0.057
39			0.045	0.048	0.050	0.057
40			0.046	0.049	0.048	0.049

FIGURE 25

Figure 2S

Figure 27

Figure 28

Lane 1: Crude Lysate
Lane 2: Flow through Lentil Chromatography
Lane 3: Wash with EMPIGEN Lentil Chromatography
Lane 4: Eluate Lentil Chromatography
Lane 5: Flow through during concentration lentil eluate
Lane 6: Pool of E1 after Size Exclusion Chromatography
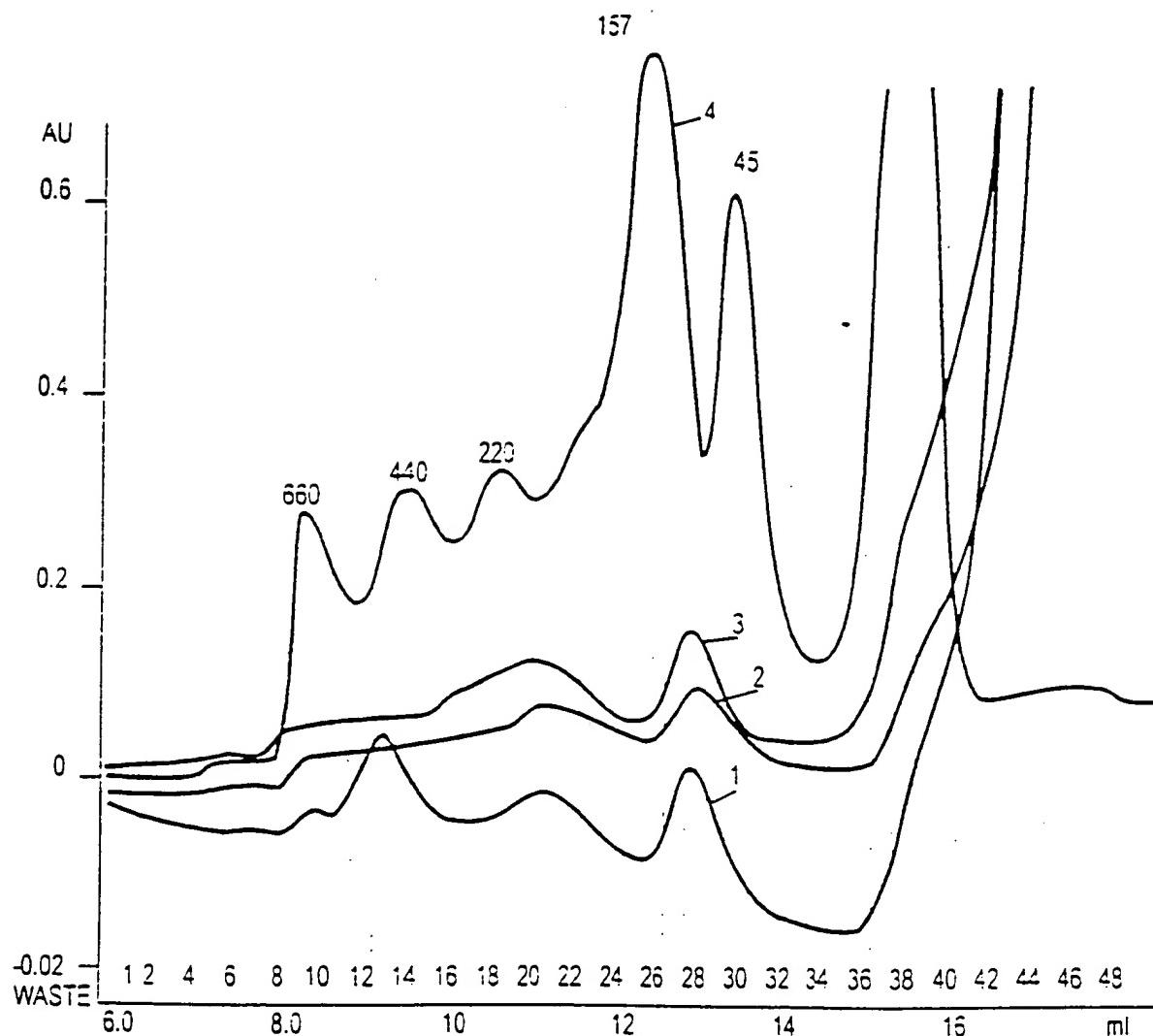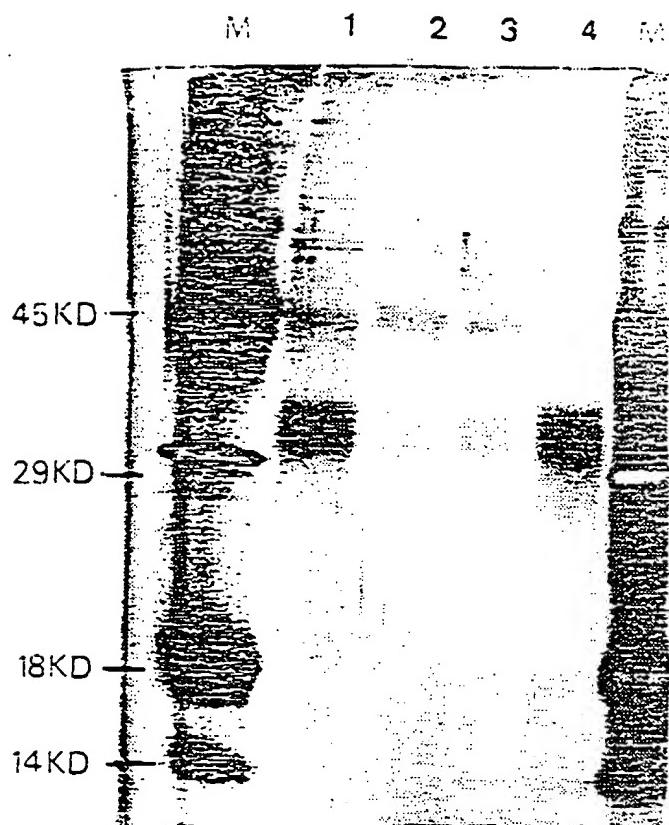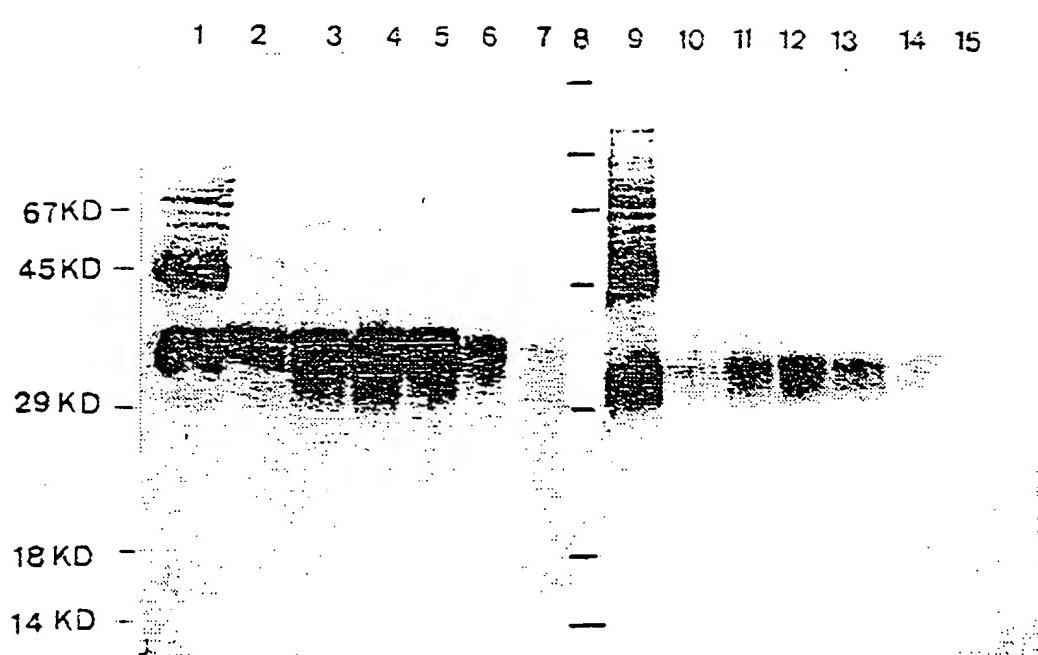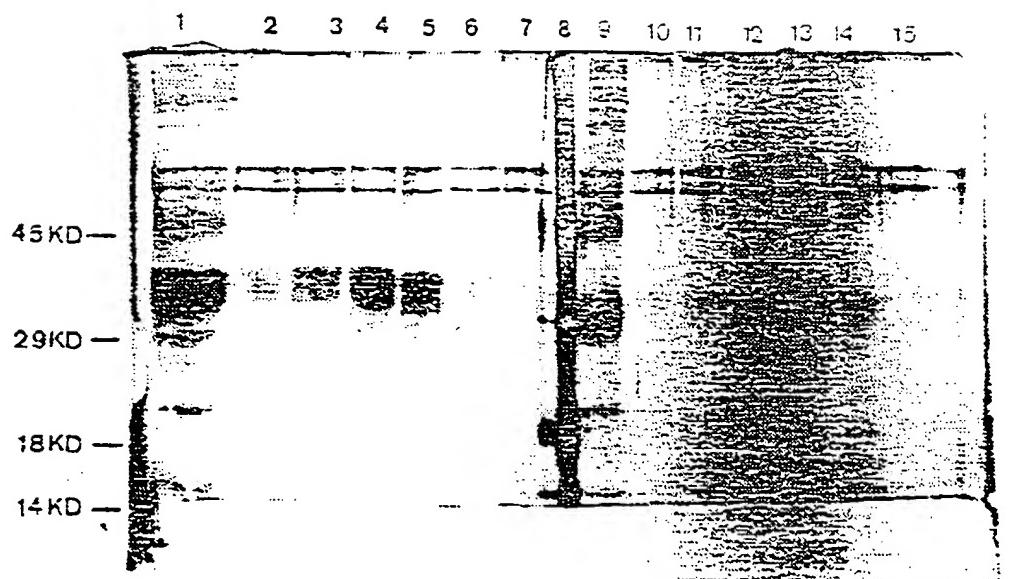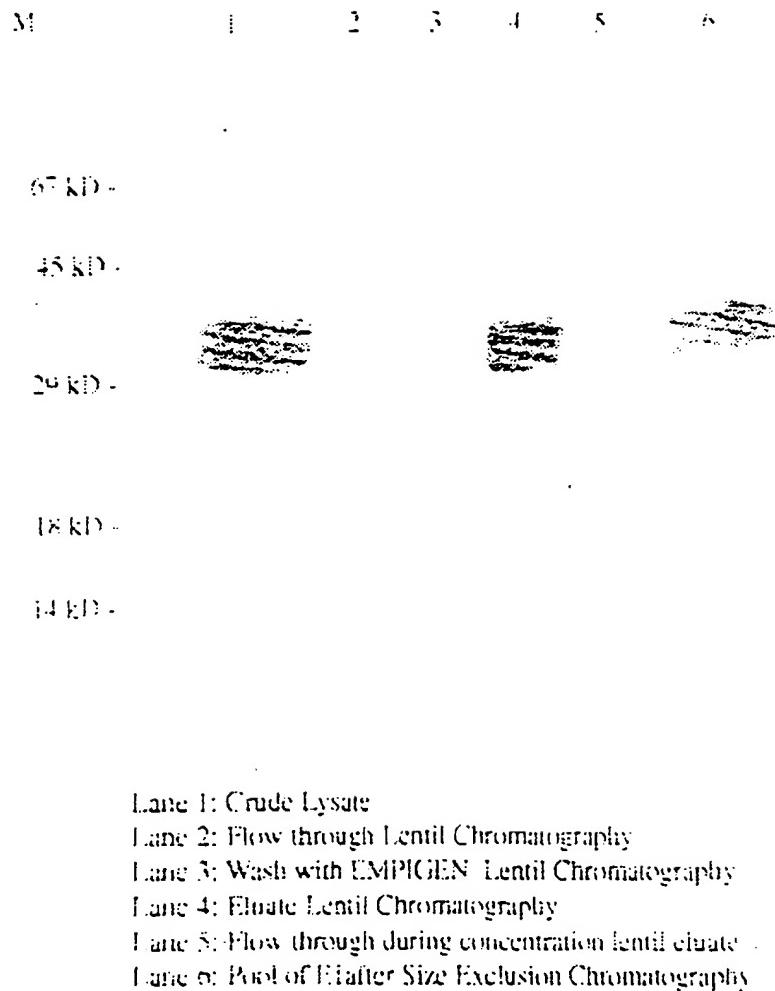
Figure 29: Western Blot Analysis with anti-E1 mouse monoclonal 5E1A10

FIGURE 30

A: NON - REDUCED

E2 + CONTAMINANTS (AGGREGATES)

B : REDUCED

CONTAMINANTS

E2 + CONTAMINANTS

POOL
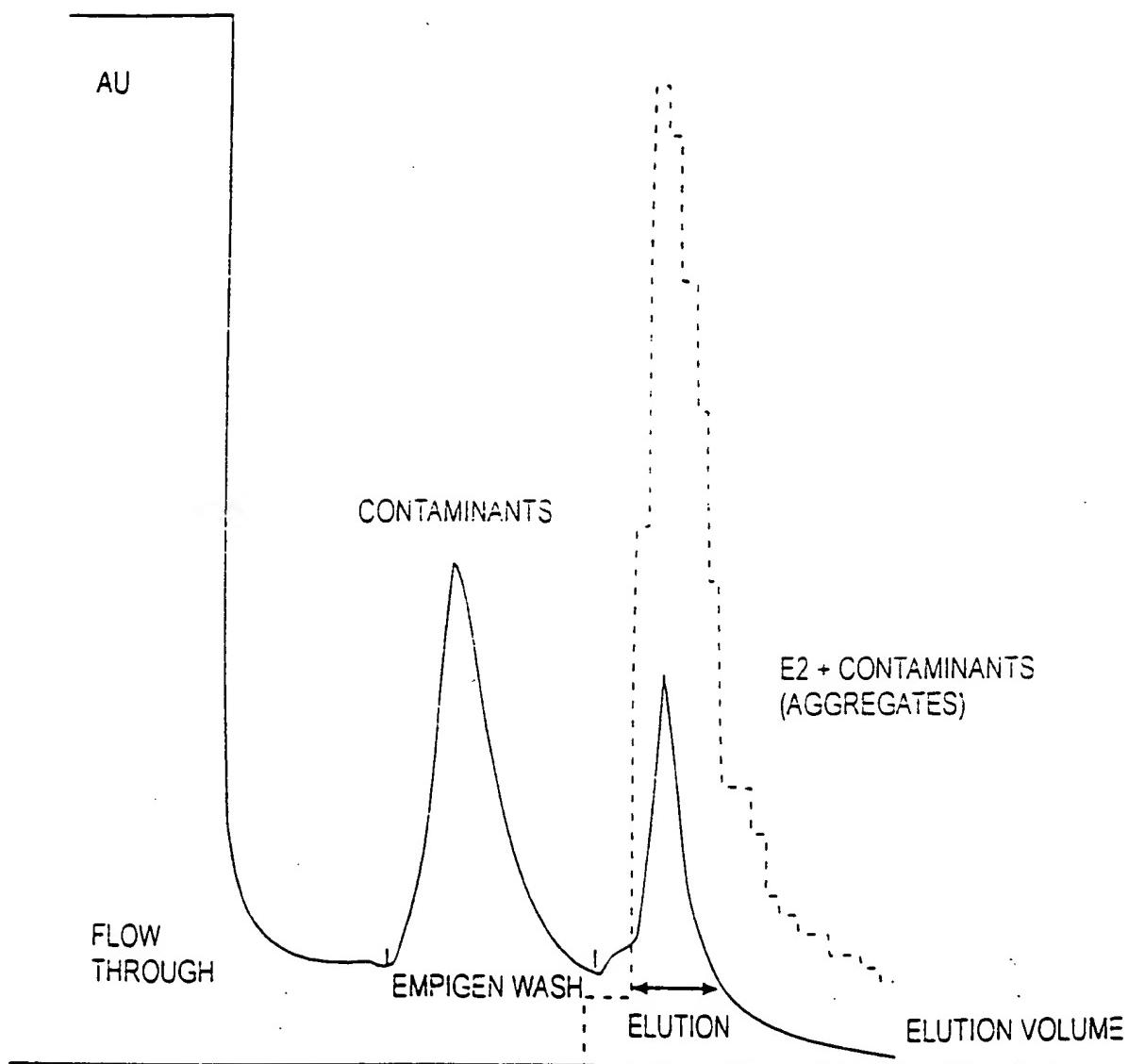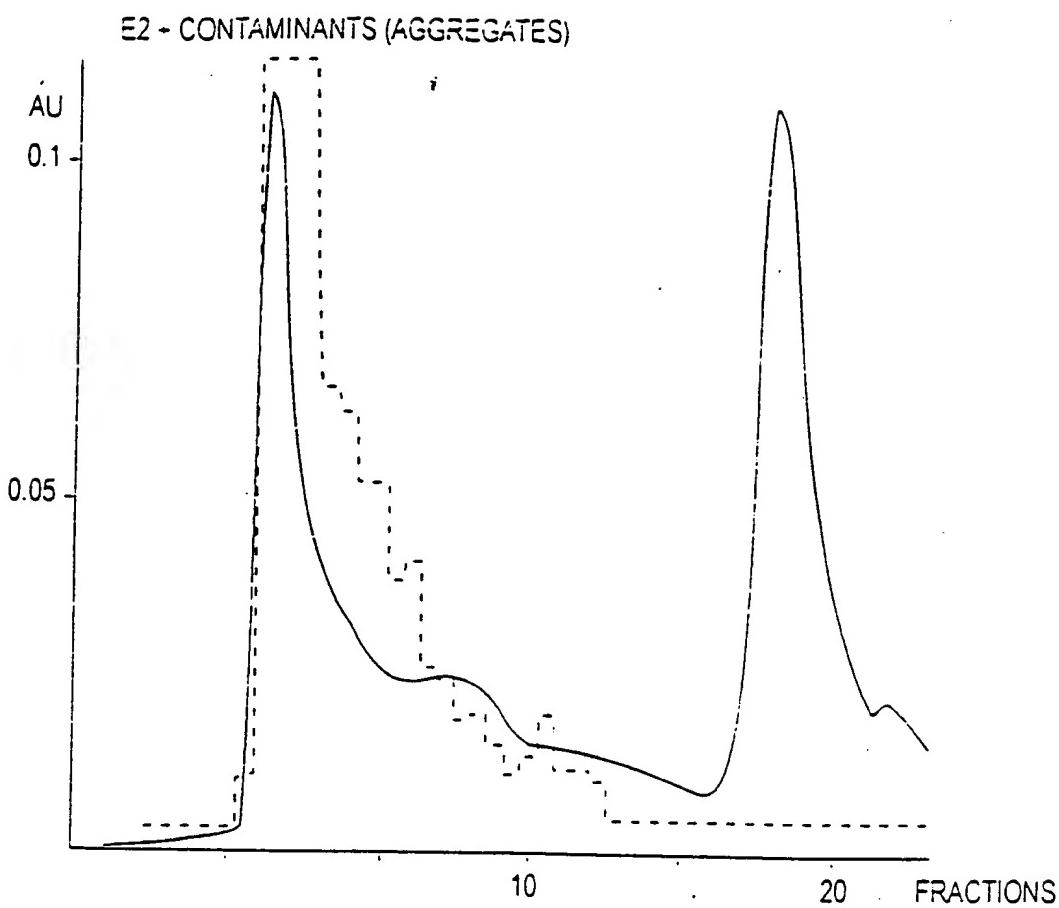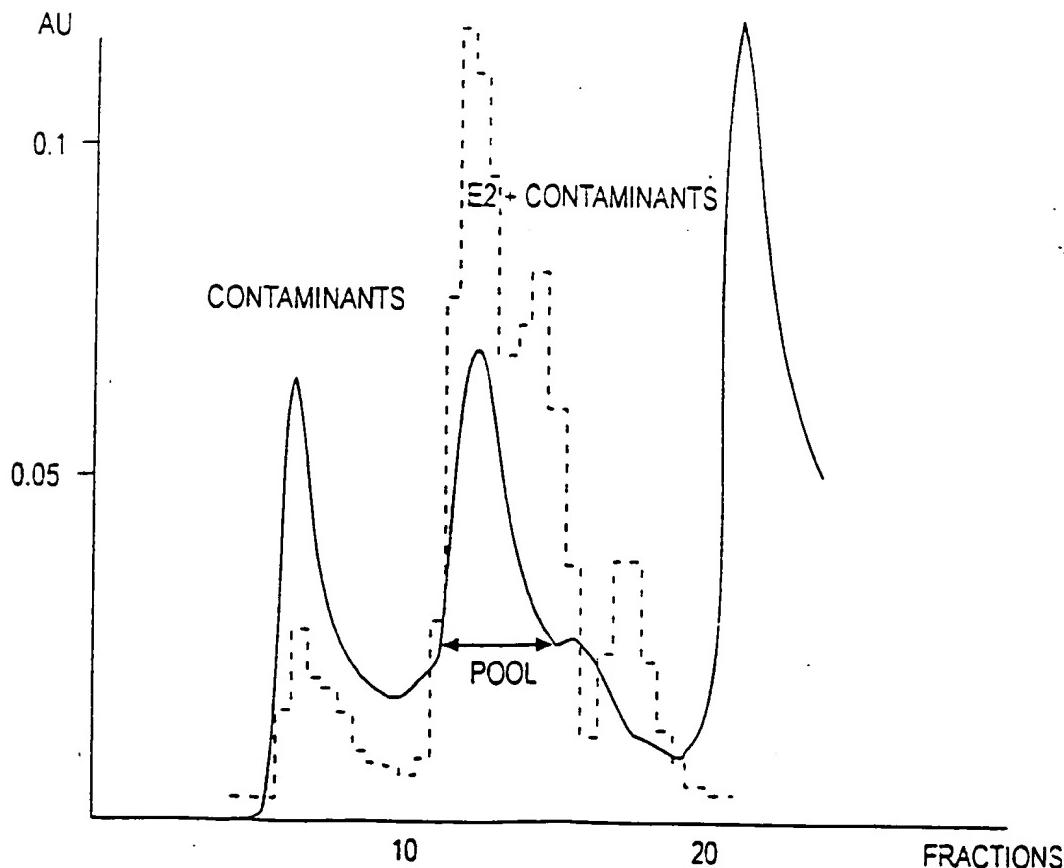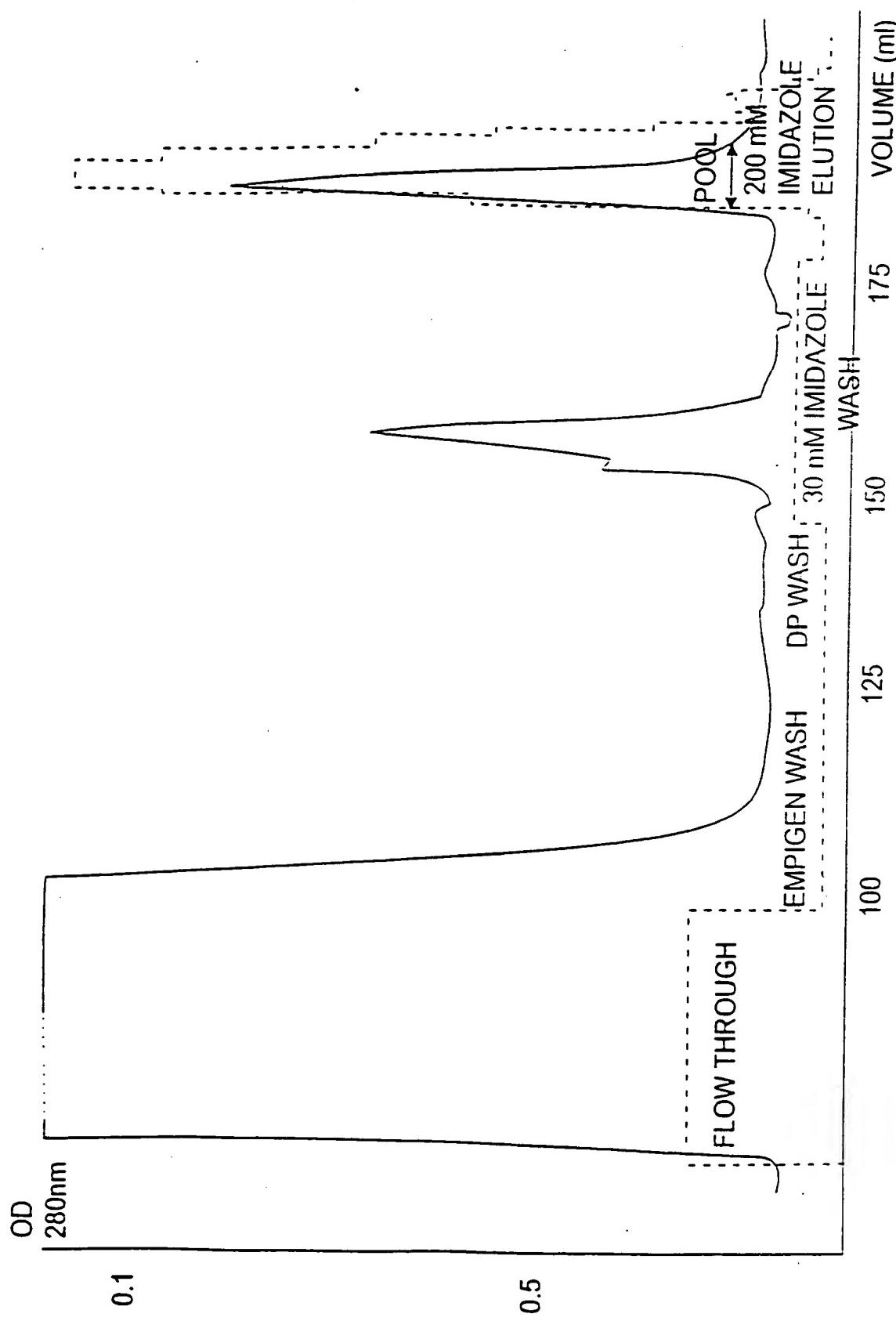
FIGURE 31

FIGURE 32

FIGURE 33:
SILVER STAIN OF PURIFIED E2

M   1   2

67 kD -

45 kD-

29 kD-

18 kD-

14 kD-
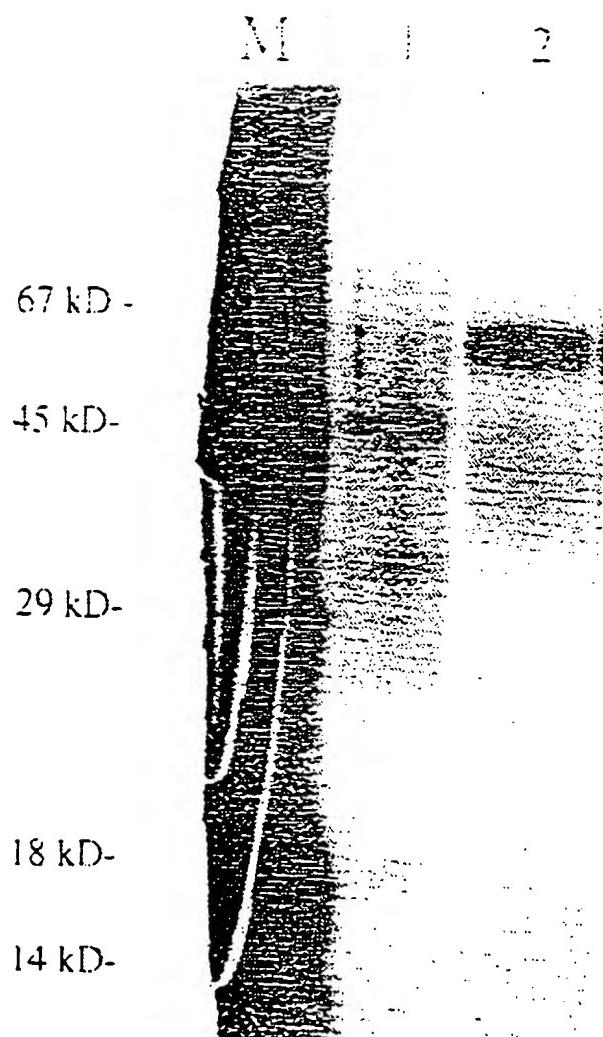
1. 30 mM IMIDAZOLE WASH Ni-IMAC
2. 0.5 ug E2

45 59 Figure 34
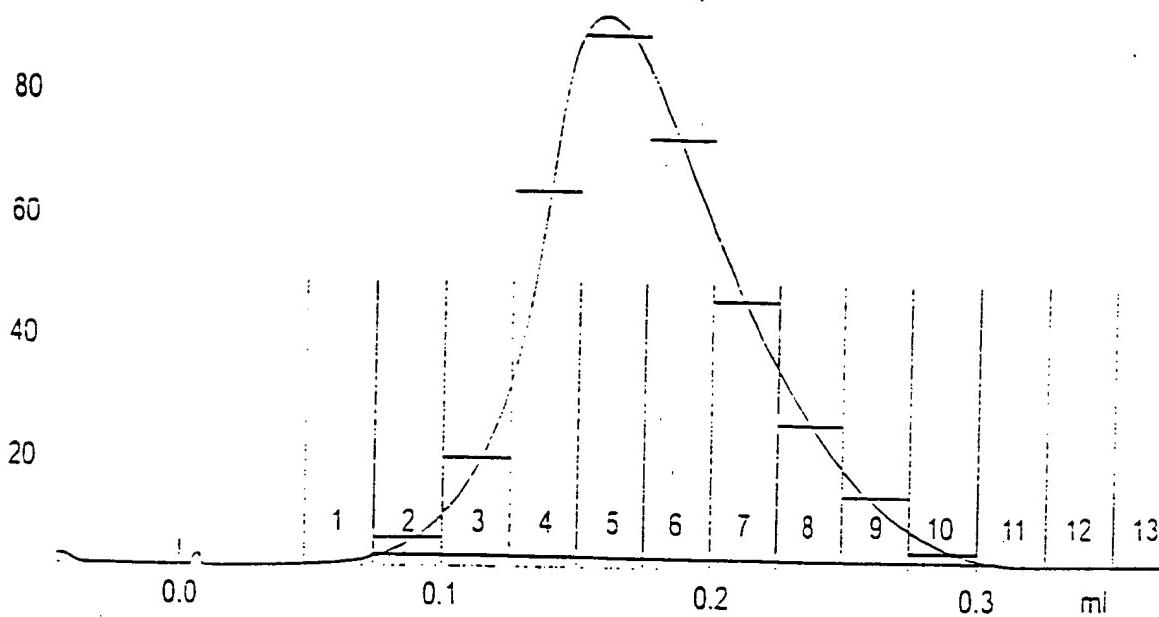
No.	Ret. (ml)	Peak start (ml)	Peak end (ml)	Dur (ml)	Area (ml*mAU)	Height (mAU)
1	-0.45	-0.46	-0.43	0.04	0.0976	4.579
2	1.55	0.75	3.26	2.51	796.4167	889.377
3	3.27	3.26	3.31	0.05	0.0067	0.224
4	3.33	3.32	3.33	0.02	0.0002	0.018

Total number of detected peaks = 4
Total Area above baseline = 0.796522 ml*AU
Total area in evaluated peaks = 0.796521 ml*AU
Ratio peak area / total area = 0.999999
Total peak duration = 2.613583 ml

FIGURE 35A

FIGURE 35B

NS4 Ab NR   NS4 Ab LTR

NS5 Ab NR   NS5 Ab LTR

E1 Ab NR   E1 Ab LTR

E2 Ab NR   E2 Ab LTR
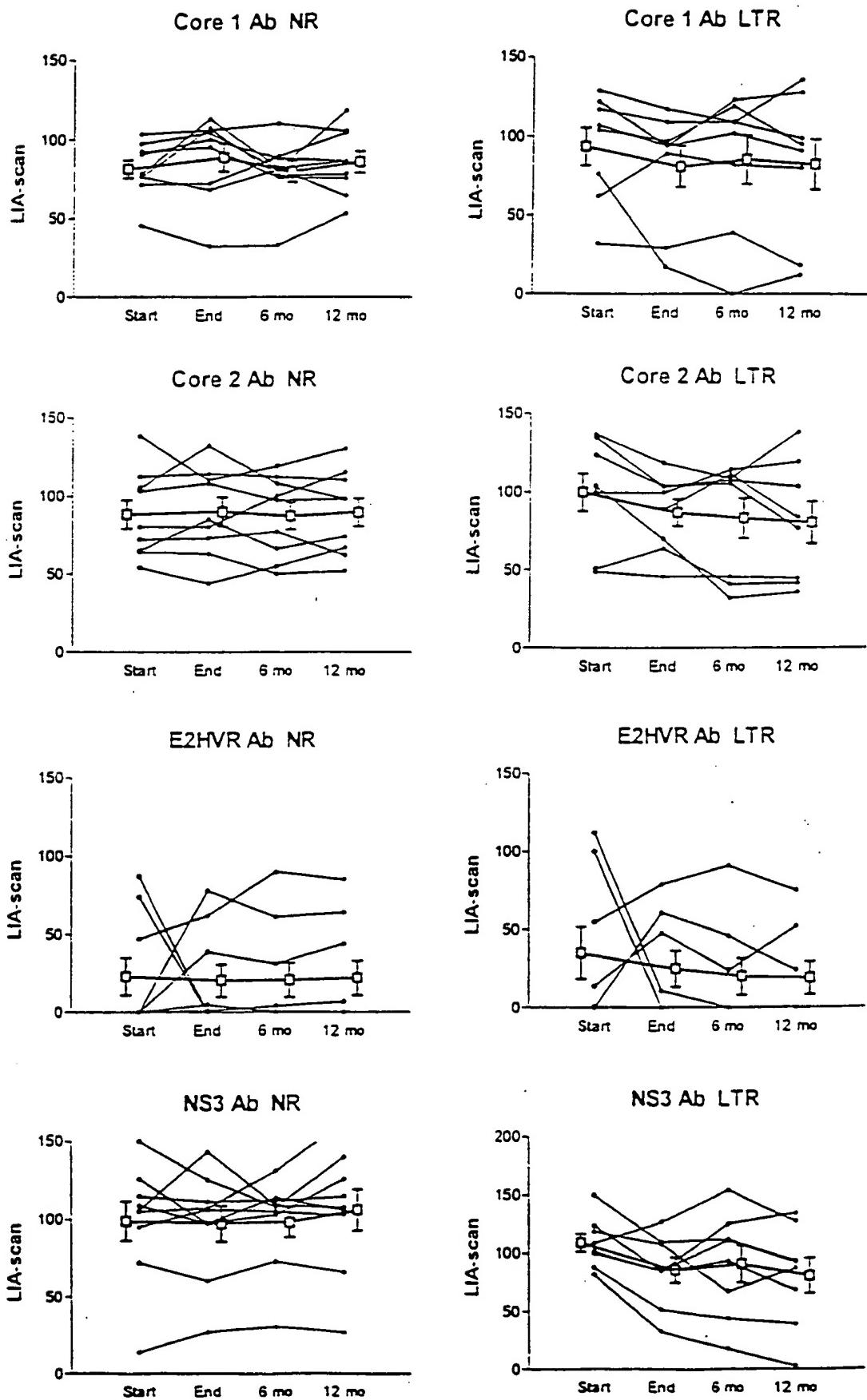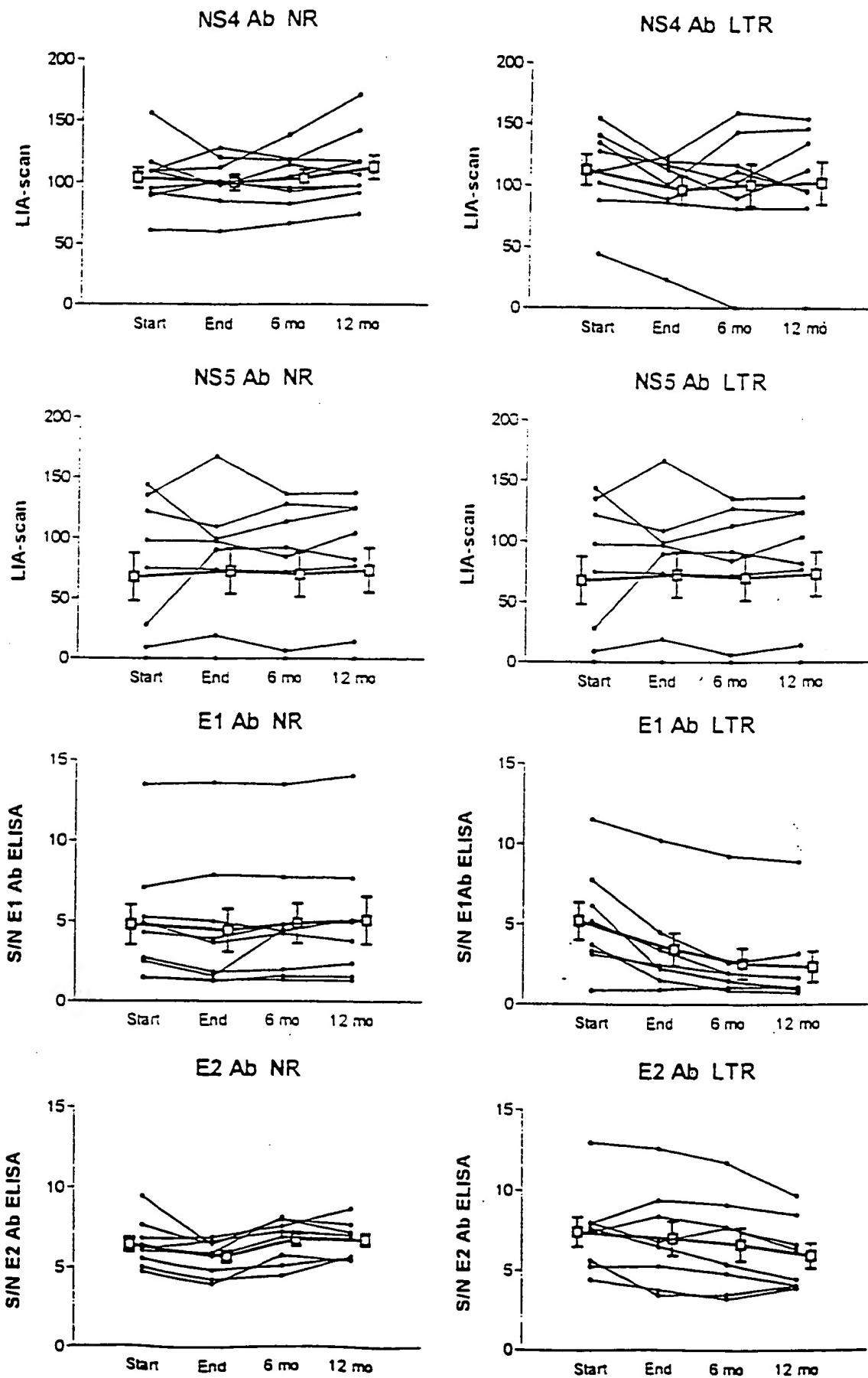
Figure 36

E1 Ab
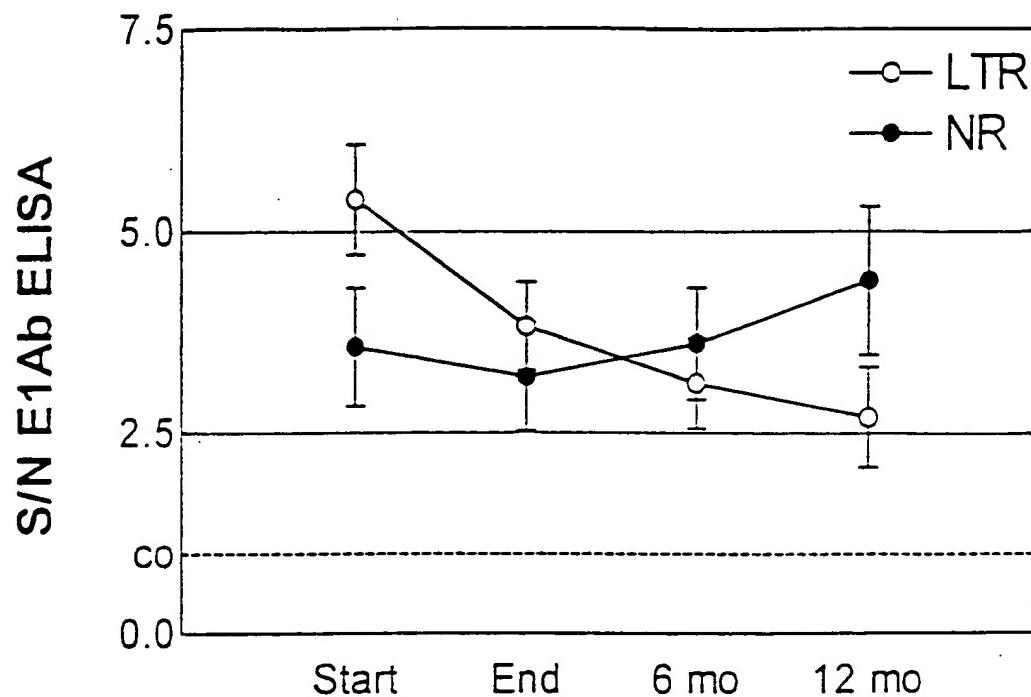
E2 Ab
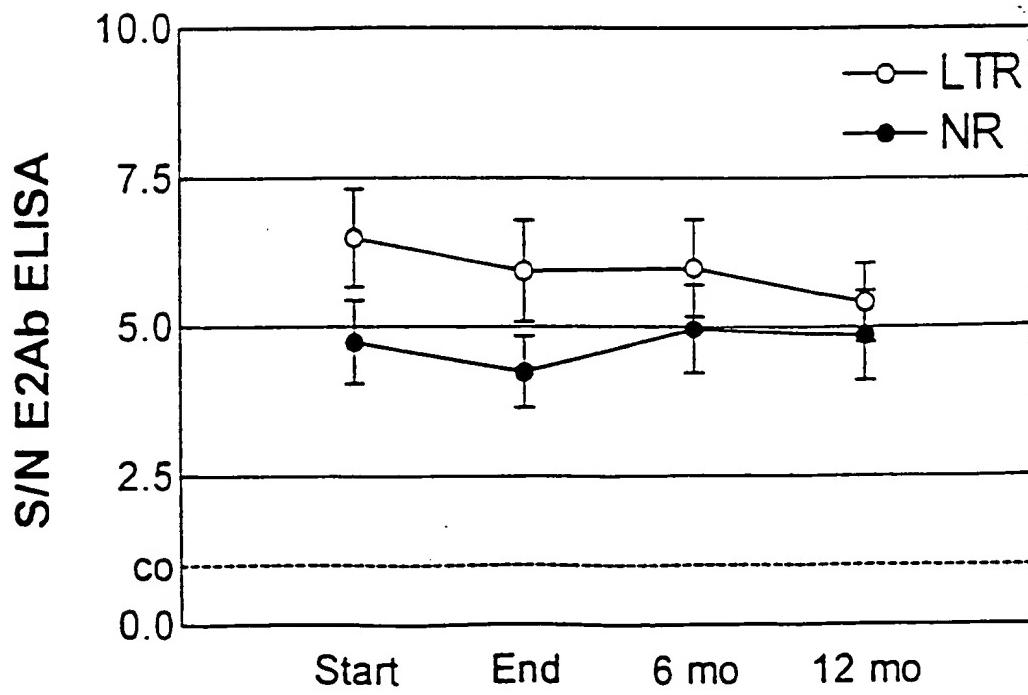
FIGURE 37
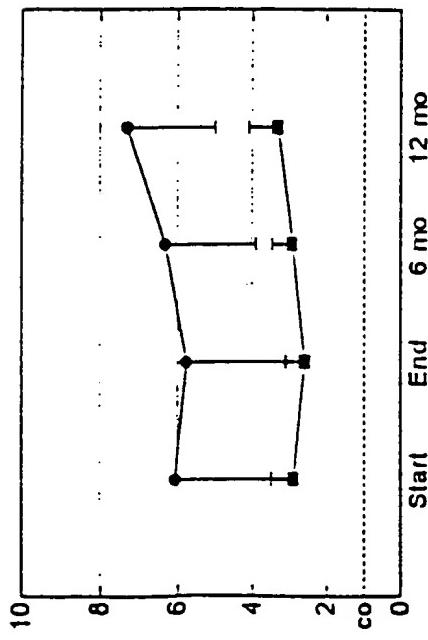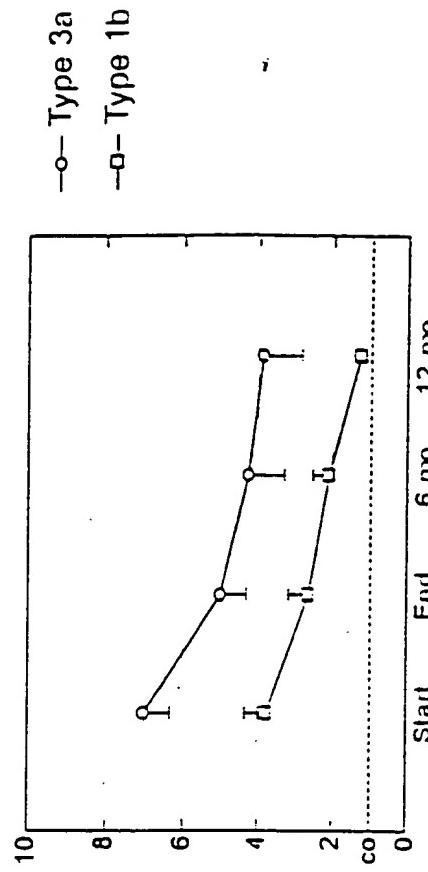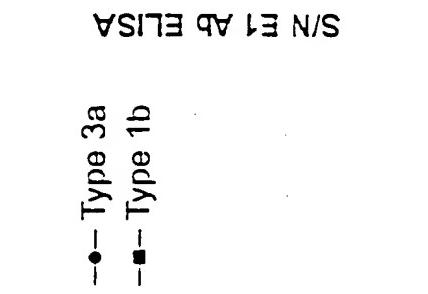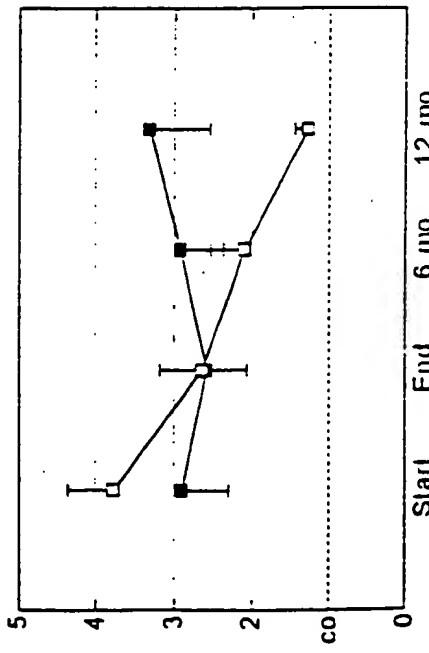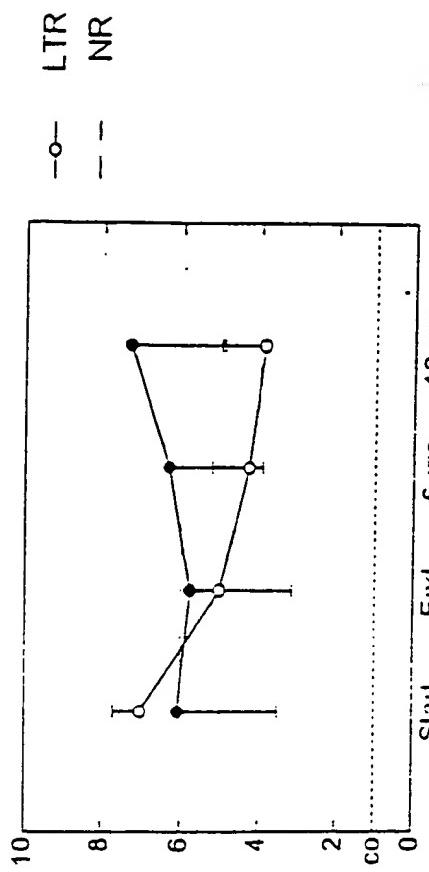
Figure 38

Relative Map Positions of anti-E2 monoclonal antibodies
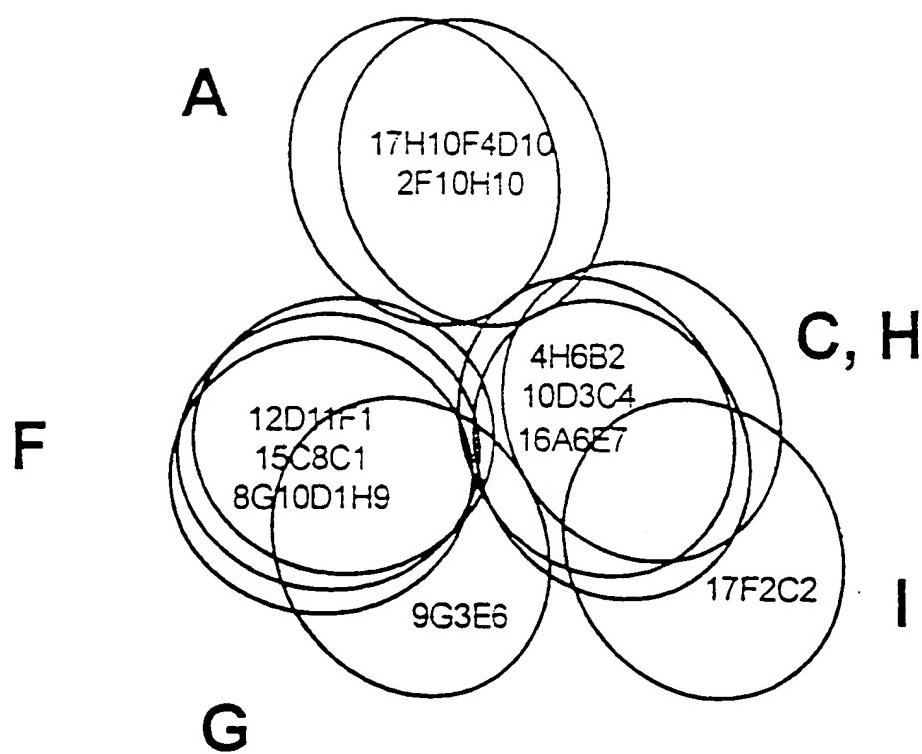
PARTIAL DEGLYCOSYLATION OF HCV E1 ENVELOPE PROTEIN
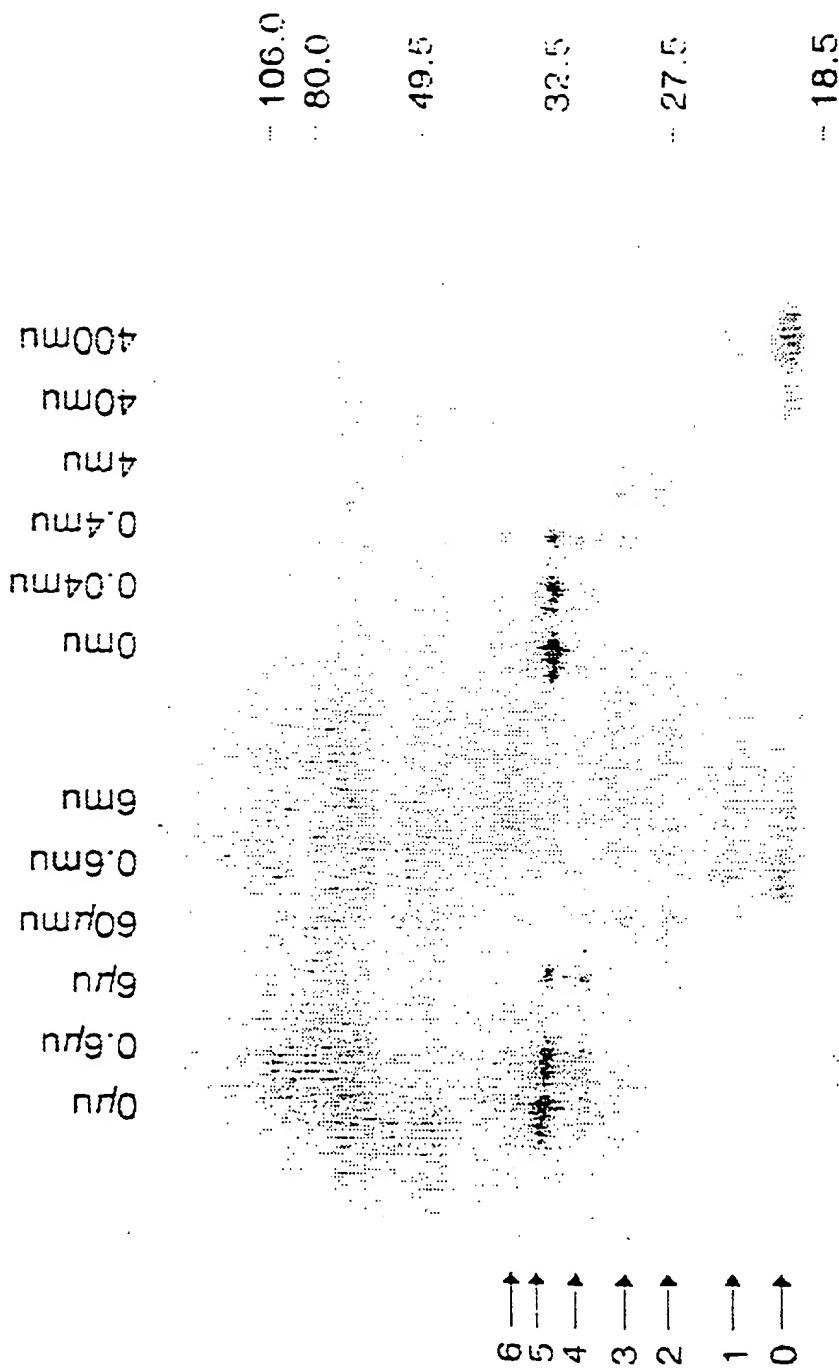
Figure 39

PARTIAL TREATMENT OF HCV E2\E2s ENVELOPE PROTEINS BY PNGase F
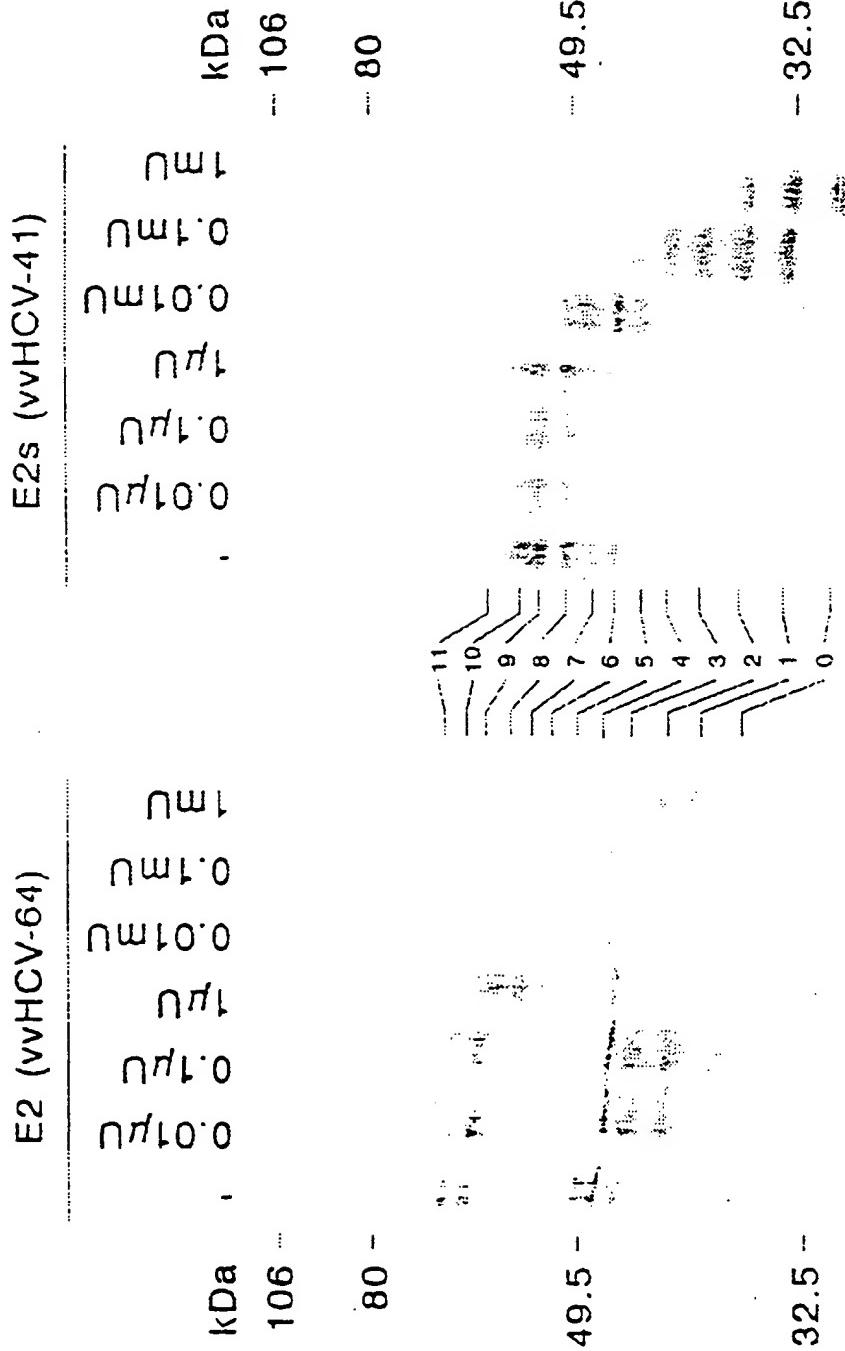
Figure 40

Fig. 41

In Vitro Mutagenesis of HCV E1 glycoprotein
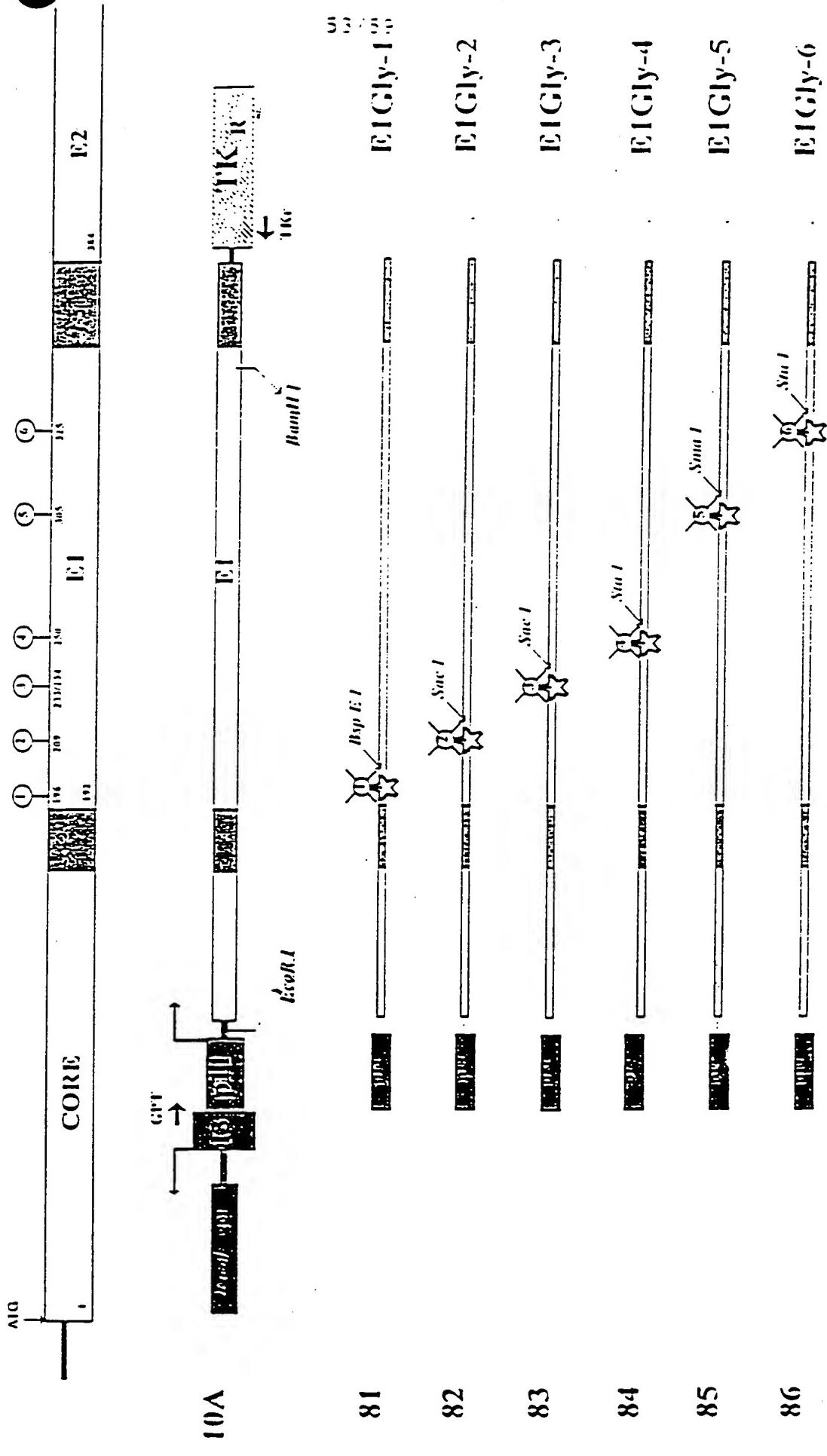
Fig. 42A   *In Vitro* Mutagenesis of HCV E1 glycoprotein

10A
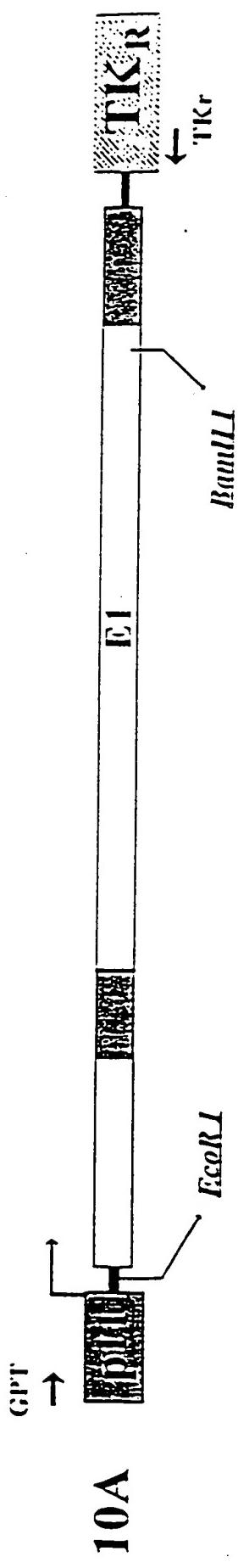
1. **First step of PCR amplification   (Gly-# and Ovr-# primers)**

30 cycles (1 min at 95°C; 1 min at 50°C; 1 min at 72°C)
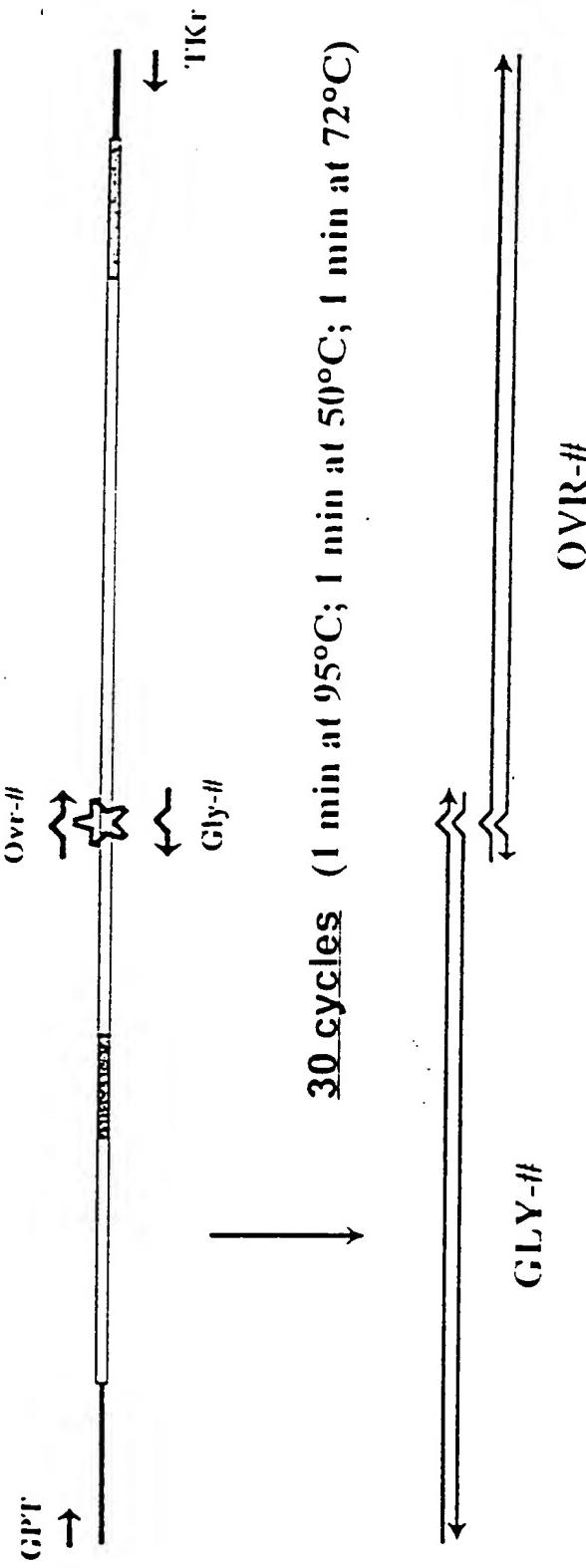
Fig. 42B

2. Overlap extension and nested PCR

a. Overlap extension

GLY-# sense strand

OVR-# antisense strand

2 cycles (1 min at 95°C; 1min at 50°C; 1 min at 72°C)
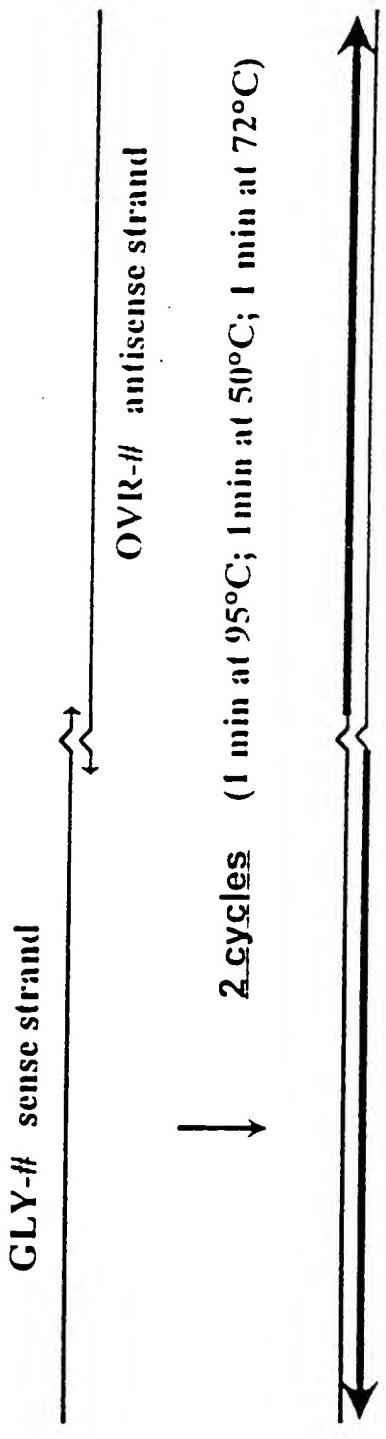
b. Nested PCR amplification (GPT-2 and TKr-2 primers)

GPT-2

TKr-2

25 cycles (1 min at 95°C; 1min at 55°C; 1 min at 72°C)

E1Gly-#

BamHI

EcoRI

Fig. 43 *In Vitro* Mutagenesis of HCV E1 glycoprotein

	HeLa cells		RK 13 cells

Figure 44A

Figure 44B

Figure 45

kDa
119
67
43
29
18

Figure 46